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THE HYDRATION OF THE ALUMINATES OF CALCIUM. II. THE HYDRATION PRODUCTS OF TRICALCIUM ALUMINATE[1]

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Abstract

Further studies on the isometric form of hydrated tricalcium aluminate are reported. These include methods of preparation, crystalline forms, X-ray diffraction pattern, and determinations of solubility and density. The products formed when the hexagonal form of hydrated tricalcium aluminate is dehydrated at definite vapor pressures of water at 21° C., were studied. The density, refractive indices, heats of solution in HCl.200 H_2O, and the X-ray diffraction patterns of several probable hydrates were determined. The experimental evidence given indicates that hydrates of $3CaO.Al_2O_3$ having 6, 8, 9½, 10½, and 12 moles of water are formed, but that the water in excess of 8 moles is very loosely held and that its removal does not materially affect the crystal structure. The exact composition of some of the higher hydrates requires confirmation.

Introduction

In a previous article (5) the preparation of a hydrate of tricalcium aluminate not previously described was reported and some of its properties were given. The hydrate was found to be easily prepared by hydrating pure tricalcium aluminate in steam under pressure, and drying the resulting product over calcium oxide. It was also prepared by treating tricalcium aluminate in a platinum dish with water at room temperature, drying the product over calcium oxide and repeating the treatment and the drying a number of times. In each case an isotropic hydrate was obtained which differed markedly from the hexagonal plates, needles and spherulites of tricalcium aluminate described by Klein and Phillips and others (2, 3, 6). The isotropic hydrate was found to contain six molecules of water of hydration. A further study of the hydrates of tricalcium aluminate is reported in this paper.

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Preparation of Pure Tricalcium Aluminate

Two samples of tricalcium aluminate were used. The preparation of Sample No. 1 which was of a very high degree of purity has already been described (5). Sample No. 3 was prepared in exactly the same way. This sample contained a very small amount of $5CaO.3Al_2O_3$ but no free lime. The chemical analyses of the two samples are given in Table I.

TABLE I

ANALYSES OF THE SAMPLES OF TRICALCIUM ALUMINATE*

	Sample No. 1	Sample No. 3	Theoretical $3CaO.Al_2O_3$
CaO	62.31	61.98	62.27
Al_2O_3	37.76	37.98	37.73
Fe_2O_3, MgO, SiO_2	absent	absent	

Analyses by David Wolochow.

A New Method of Preparing the Hexahydrate of Tricalcium Aluminate

As reported in the first paper of this series (5) it has been found that on heating at 600 to 900° C., hydrated tricalcium aluminate is decomposed to $5CaO.3Al_2O_3$ and free calcium oxide. It was also reported that when the resulting mixture was placed in saturated steam at 150°C. recombination took place and the hexahydrate of tricalcium aluminate was again formed. This suggested that possibly hydrated tricalcium aluminate might be formed directly from lime and alumina, if a mixture of the correct composition were exposed to steam at 150° C. This was actually found to be the case, thus providing a very simple method of preparing the hexahydrate of tricalcium aluminate and obviating the necessity of first preparing the anhydrous compound. As ignition of the hydrate at about 1100°C. produces anhydrous tricalcium aluminate, the method may also be used to prepare the latter substance. This is much simpler than the usual procedure.

When pure tricalcium aluminate is exposed to steam at 150° C. the crystals of the hexahydrate take the form of trapezohedrons. When this hydrate is prepared by exposing a mixture of lime and alumina to saturated steam at the same temperature, cubes—and more rarely dodecahedrons—are formed. In each case the refractive index is the same, namely $N_{Na} = 1.604 \pm 0.002$, and the X-ray patterns are also identical. Fig. 1 and Fig. 2 are photomicrographs of crystals of hydrated tricalcium aluminate prepared from tricalcium aluminate, Sample No. 1, by hydration on a slide in a thermostat at 25.5° C. Fig. 3 is a photomicrograph of crystals of the hexahydrate of tricalcium aluminate prepared by heating in saturated steam at 150° C., a 3:1 mixture of lime (CaO) and alumina (Al_2O_3).

FIG. 1. *The Hexahydrate of Tricalcium Aluminate Showing Trapezohedrons.* × *360.*

FIG. 2. *The Hexahydrate of Tricalcium Aluminate Showing Trapezohedrons.* × *360.*

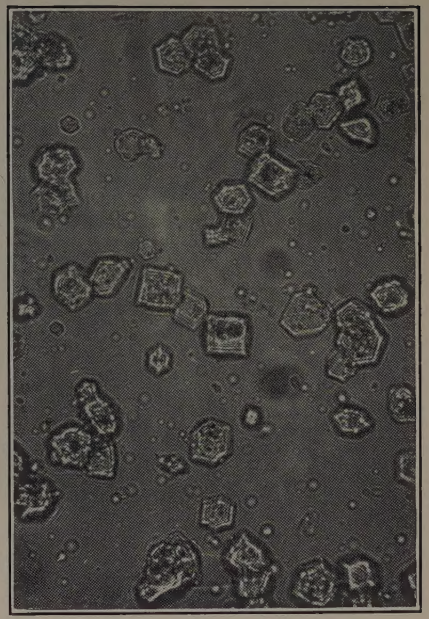

FIG. 3. *The Hexahydrate of Tricalcium Aluminate Showing Cubes.* × *360.*

PLATE I

The Solubility of the Hexahydrate of Tricalcium Aluminate.

The sample of $3CaO.Al_2O_3.6H_2O$ used was prepared from tricalcium aluminate, Sample No. 1, by hydration in water and drying to constant weight over calcium oxide. The solubility determinations were made in gold-lined tubes, the liquid coming in contact only with gold. Precautions were also taken to prevent any contamination with carbon dioxide. It was found that considerable supersaturation was at first produced and that equilibrium was attained very slowly. By washing the hydrate several times in the tube the smaller particles were all removed and a constant value was obtained for the solubility. The tubes were centrifuged before samples of the liquid were withdrawn. After equilibrium had been attained at 21°, 25°, 30° and 40° C., respectively, titrations of the aqueous solution with N/50 hydrochloric acid, using phenolphthalein and methyl orange as indicators, indicated that the quantities of lime and alumina in solution were in all cases in the ratio of $3CaO$ to Al_2O_3. Gravimetric determinations confirmed this. The saturated solution at 21° C. had a pH of 11.00. From the gravimetric determinations the solubility of the hexahydrate at 21° and 40° C., respectively, was found to be equivalent to 0.0246 and 0.0268 gm. of anhydrous tricalcium aluminate per 100 cc. of solution.

The Density of the Hexahydrate.

Two values for the density of the hexahydrate of tricalcium aluminate were obtained by the displacement method in purified and redistilled kerosene. After the hydrate had been suspended in the kerosene, special precautions were taken to remove all the air from it by means of a vacuum pump. The sample used in the first determination was prepared from tricalcium aluminate, Sample No. 1, by treatment with saturated steam at 150° C. The sample used in the second determination was also prepared from Sample No. 1, by hydration in water at room temperature. Both samples of the hydrate were dried to constant weight over calcium oxide. The results are given in Table II.

TABLE II

DENSITY OF $3CaO.Al_2O_3.6H_2O$ AT 20.0° C.

Exp. No.	Weight of hexahydrate in grams	Water as per cent of anhydrous aluminate	Density of kerosene in grams per cc.	Kerosene displaced in grams	Density of $3CaO.Al_2O_3.6H_2O$ in grams per cc.
1	2.3393	39.97	0.8094	0.7506	2.5224
2	2.1549	40.08	0.8094	0.6918	2.5212
				Mean density	2.522

X-ray Diffraction Pattern of the Hexahydrate of Tricalcium Aluminate.

The photographs of the X-ray diffraction patterns were obtained with a multiple powder spectrograph manufactured by the General Electric Company. A Coolidge tube with a water-cooled molybdenum target was used. The

accuracy of the readings was checked by means of patterns obtained simultaneously with pure sodium chloride. The planar spacings given in the first column of Table III were obtained by direct measurement. The estimated relative intensity is given in the second column. Values of $\sin^2\theta$ and the observed ratio of $\sin^2\theta$ were calculated from the planar spacings and the reflecting planes identified graphically by the method of Hull and Davey (1). The lattice constant, as given in the last column, was calculated from the observed planar spacings and the relative spacing.

TABLE III

DATA FROM POWDER PHOTOGRAPHS OF $3CaO.Al_2O_3.6H_2O$

Planar spacing d_{hkl} in Å	Estimated relative intensity*	$\sin^2\theta$	Ratio, $\sin^2\theta$		Miller indices	Relative spacing	Lattice constant a_o in Å
			Observed	True			
5.13	S	0.00479	6.01	6	112(1)	0.4083	12.566
4.45	S	.00635	7.97	8	110(2)	.3535	12.587
3.36	S	.01110	13.93	14	123(1)	.2673	12.572
3.14	S	.01278	16.04	16	100(4)	.2500	12.560
2.814	SS	.01592	19.97	20	120(2)	.2236	12.585
2.68	WW	.01755	22.02	22	233(1)	.2132	12.570
2.57	W	.01908	23.95	24	112(2)	.2041	12.590
2.47	M	.02065	25.92	26	150(1)		
					134(1)	.1961	12.595
2.30	SS	.02382	29.90	30	125(1)	.1826	12.597
2.04	SS	.03028	38.00	38	116(1)		
					235(1)	.1622	12.575
1.99	WW	.03182	39.94	40	130(2)	.1581	12.586
1.815	W	.03826	48.01	48	111(4)	.1443	12.575
1.742	S	.04153	52.12	52	230(2)	.1387	12.562
1.710	W	.04310	54.08	54	112(3)		
					127(1)	.1361	12.566
1.679	S	.04471	56.10	56	123(2)	.1336	12.564
1.595	M	.04954	62.17	62	156(1)		
					237(1)	.1270	12.559
1.572	M	.05100	64.00	64	100(8)	.1250	12.573
1.484	W	.05723	71.82	72	110(6)		
					114(2)	.1178	12.592
1.407	S	.06366	79.90	80	120(4)	.1118	12.585
1.372	M	.06695	84.02	84	124(3)	.1091	12.574
1.342	M	.06998	87.82	88	233(2)	.1066	12.589
1.298	W	.07480	93.87	94	239(1)	.1031	12.585
1.283	W	.07656	96.08	96	112(4)	.1021	12.571
1.244	W	.08144	102.2	102	277(1)	.0990	12.564
1.199	M	.08766	110.0	110	259(1)	.0953	12.575
1.167	M	.09254	116.1	116	250(2)	.0928	12.569
1.148	M	.09563	120.0	120	125(2)	.0913	12.576
1.120	M	.10047	126.1	126	123(3)	.0891	12.572
1.086	W	.10686	134.1	134	279(1)	.0864	12.571
1.048	W	.11475	144.0	144	100(12)		
					122(4)	.0833	12.576
1.020	M	.12113	152.0	152	116(2)		
					235(2)	.0811	12.575
						Mean $a_o = 12.576Å \pm 0.02$	

SS, very strong; S, strong; M, medium; W, weak; WW, very weak.

Interpretation of the Pattern Obtained from the Hexahydrate of Tricalcium Aluminate ($3CaO.Al_2O_3.6H_2O$).

The hexahydrate of tricalcium aluminate crystallizes in the form of trapezohedrons and more rarely as cubes and dodecahedrons, depending on the method of preparation. The crystals therefore belong to the normal or holohedral class of the isometric system. The X-ray pattern shows lines corresponding to the requirements of a body-centered lattice. Of the two possible holohedral space groups built upon this lattice, the absence of reflections from such planes as 130(1), 110(3), 110(4), 334(1), 145(1), 113(2), etc. makes it probable that Oi-10 is the correct one. At the same time, the agreement of the pattern with the more or less complicated requirements of Oi-10 strengthens the symmetry assignment to the holohedral class by making unlikely the other space groups that are built on the body-centered lattice. The lattice constant determined by measurement of planar spacings is found to be $12.576\text{Å} \pm 0.02$ and there are eight molecules in the unit cube. Using these values in the equation $D = \frac{mM}{a_0^3}$ the density was calculated to be 2.53 which corresponds closely to the determined value of 2.52.

Preparation of Higher Hydrates of Tricalcium Aluminate

When tricalcium aluminate is shaken with excess of water at a fairly low temperature, a hydrate having the crystalline form described by Klein and Phillips (2) is readily formed. If the hydrated material is to be free from the isometric form, it is necessary to prevent the heat generated during hydration from raising the temperature. It was found that, when the following method was used, a product composed entirely of hexagonal plates, needles and spherulites of hydrated tricalcium aluminate was obtained.

Freshly ignited tricalcium aluminate was gradually added to ice-cold carbon-dioxide-free water contained in a platinum dish cooled by a mixture of ice and water. During hydration, any lumps formed were from time to time broken up. The material was kept in contact with water and protected from the carbon dioxide of the air at a temperature not above 21° C. for about two weeks.

Samples of this hydrated material contained in platinum crucibles were then placed in evacuated vessels over saturated solutions of various salts,—a large excess of the salt being present—until equilibrium had been attained, as shown by constancy of weight. In some cases solutions of sulphuric acid were used. The temperature was maintained at 21° C. The conditions of exposure are given in Table IV.

TABLE IV

SYSTEMS USED FOR OBTAINING CONSTANT HUMIDITY

System	Per cent humidity	Vapor tension at 21° C., in mm. Hg.
$Pb(NO_3)_2 - H_2O$ (saturated)	98	18.1
$KCl - H_2O$ (saturated)	87	16.1
$CH_3COOK - H_2O$ (saturated)	20	3.7
$H_2SO_4(63\%) - H_2O(37\%)$		2.2
$H_2SO_4(70.4\%) - H_2O(29.6\%)$		0.8
$CaO - Ca(OH)_2$		0.2

Table V gives the percentage of water held by the hydrated tricalcium aluminate at equilibrium under the conditions stated. The amount of water retained was determined by ignition of the sample at 1100° C. for two hours, and is expressed as a percentage of the anhydrous tricalcium aluminate.

TABLE V

COMPOSITION OF HYDRATED TRICALCIUM ALUMINATE DRIED TO CONSTANT WEIGHT AT DEFINITE VAPOR TENSIONS OF WATER AT 21°C.

Exp. No.	$3CaO.Al_2O_3$ Sample No.	Weight of anhydrous $3CaO.Al_2O_3$ in grams	Weight of hydrated $3CaO.Al_2O_3$ in grams	Water held, per cent of anhydrous weight	Average water held, per cent.	Moles H_2O per mole $3CaO.Al_2O_3$
A. Over a Saturated Solution of Lead Nitrate (18.1 mm.)						
1	3	0.3095	0.5621	81.6		
2	3	0.3157	0.5841	85.0	83.3	12.5
B. Over a Saturated Solution of Potassium Chloride (16.1 mm.)						
3	1	0.2905	0.5016	72.7		
4	1	0.3022	0.5213	72.5	72.6	10.88
C. Over a Saturated Solution of Potassium Acetate (3.7 mm.)						
5	3	0.3157	0.5377	70.3		
6	3	0.3095	0.5266	70.1		
7	3	0.2721	0.4633	70.3		
8	3	0.2823	0.4802	70.1	70.2	10.52
D. Over 63% H_2SO_4 (2.2 mm.)						
9	3	0.2614	0.4273	63.5		
10	3	0.2428	0.3985	64.1	63.8	9.58
E. Over 70.4% H_2SO_4 (0.8 mm.)						
11	3	0.2614	0.4250	62.6		
12	3	0.2428	0.3962	63.1	62.8	9.42
F. Sample Dried over CaO.						
13	3	0.4171	0.6391	53.0		
14	3	0.3414	0.5213	52.7		
15	3	0.3591	0.5524	53.8		
16	3	0.3509	0.5405	54.0		
17	1	0.2843	0.4338	52.6		
18	1	0.1547	0.2376	53.4	53.25	7.99

The results given in Table V indicate that tricalcium aluminate probably forms a series of hydrates in addition to the isometric hexahydrate. The decision as to the exact composition of these hydrates presents some difficulty. It was found that both rise in temperature and pressure favored the transformation of the hexagonal form to the isometric hydrate. Even the grinding of a sample of the hexagonal hydrates in a mortar sometimes caused partial transition to the isometric form. Great care must therefore be taken if one is to obtain homogeneous samples free from the hexahydrate.

The lowest temperature at which transition from the hexagonal to the isometric form was observed at atmospheric pressure was 25° C., but some samples kept at this temperature for long periods still contained the two forms. When samples of the hexagonal form of hydrated tricalcium aluminate, which had been dried to constant weight at various tensions of water vapor at 21° C. (Table V), were further dried in an oven at 100° C., the material containing the highest percentage of water before the final drying, lost water until it contained less than any of the other samples. This is probably due to partial transition to the isometric form, the change taking place more readily as the initial percentage of water in the material increases. The behavior of samples of varying water content on drying at 100° C. is recorded in Table VI.

TABLE VI

BEHAVIOR OF THE HEXAGONAL FORM OF HYDRATED TRICALCIUM ALUMINATE ON DRYING IN AIR AT 100° C.

Exp. No.	$3CaO.Al_2O_3$ Sample No.	Weight of Anhydrous $3CaO.Al_2O_3$ in grams	Weight of hydrated sample after drying at 100°C. in grams	Per cent H_2O before heating at 100° C.	Per cent H_2O after heating at 100° C.	Time of drying at 100° C. in hours
1	3	0.1955	0.2837	80.3	45.1	48
2	3	0.2747	0.4027	77.4	46.7	29
3	3	0.3252	0.4771	77.6	46.7	29
4	1	0.3022	0.4487	72.5	48.4	72
5	1	0.2721	0.4032	70.3	48.2	20
6	1	0.2114	0.3189	51.7	50.9	20

Adsorption of water vapor by the finely crystalline hydrate also causes some uncertainty as to the exact formula of the higher hydrates. The amount of water vapor held in this way at a definite aqueous tension of the atmosphere to which the sample is exposed, varies with the fineness of subdivision, and this may give rise to variations in the results obtained with different samples prepared from the same material.

The behavior of a substance of known composition when dried under similar conditions may indicate to what extent adsorption of water by the samples described in Table V may be expected. Thorvaldson and Brown (4) found that finely crystalline calcium hydroxide (prepared by exposing calcium oxide to steam at 150° C.) when dried in a vacuum over calcium oxide attained in two or three days a water content within 0.05% of the theoretical value for $Ca(OH)_2$. When samples of calcium hydroxide prepared in this way were exposed over a saturated solution of $(NH_4)_2SO_4$ at 21° C. (aqueous tension 15.0 mm.), they adsorbed 0.1 to 0.6% water, according to the size of the crystals.

Calcium oxide, hydrated in water vapor at room temperature, formed a product which was apparently amorphous when examined under the microscope and was found to retain an excess of about 0.4% water[1] after drying for about

[1] *All percentages of water are calculated as per cent of anhydrous sample of CaO.*

five days over lime. Similarly prepared samples containing an excess of 16% water lost all but 2.5% of this excess in nine days when placed in an atmosphere of 81% humidity at 21° C. After drying over calcium oxide, these samples were again placed in an evacuated vessel over a saturated solution of ammonium sulphate at 21°C. (aqueous tension 15.0 mm.). They were found to approach an equilibrium in 60 hr. when they contained an excess of 1.9% water.

If crystalline hydrated tricalcium aluminate used in this series of experiments possessed a capacity for adsorption of water vapor similar to that of the crystalline calcium hydroxide described above, one would not expect any appreciable adsorption of water vapor for the samples dried over lime; and only a very small fraction of one per cent would be expected in the case of the material exposed over potassium acetate (aqueous tension 3.7 mm.). Even were the hydrated tricalcium aluminate to possess as great a power of adsorption of water vapor as the calcium hydroxide prepared by hydration at room temperature, one would expect it to hold only about 2% of water when in equilibrium with a saturated solution of potassium chloride (aqueous tension 16.1 mm.) and much less at lower vapor tensions.

Experiments with the isotropic form of tricalcium aluminate showed that the hydrate formed in steam at 150° C., when exposed over a saturated solution of lead nitrate at 21° C. (aqueous tension 18.1 mm.), retained after three days exposure only 2.5% water in excess of that required for the hexahydrate. After an exposure of 24 hr. over a saturated solution of potassium chloride (aqueous tension 16.1 mm.) the excess of water held was only one per cent. Experiments in which samples of the hexahydrate prepared at room temperature were wetted and then placed over the above solutions gave similar results.

It seems therefore entirely improbable that the series of products which appear to be in equilibrium with water vapor at definite tensions (Table V) can be explained by the assumption of adsorption of water vapor by a single hydrate of the hexagonal form. However, it is not easy to decide with certainty what hydrates of definite composition exist. The behavior of the hexagonal material during gradual dehydration in equilibrium with the aqueous vapor of the solutions mentioned in Table IV, provides some further evidence as to the probable composition of the successive hydrates formed.

When at 21° C. the vapor tension is kept constant at 18.1 mm., which is only 0.4 mm. below the vapor tension of water at that temperature, the wet material at first loses water rather rapidly and then gradually reaches a constant water content of about 83%[1]. This represents a composition of approximately $3CaO.Al_2O_3.12.5H_2O$, but as a considerable adsorption of water must take place in this case it is more likely that the hydrate contains only 12 moles of water of hydration.

When a vapor tension of 16.1 mm. is used the content of water in the material drops fairly rapidly to 72.5%, while lowering the tension to 3.7 mm. causes a further loss of only about 2%. When material which has already been

[1] *The percentage of water is in all cases expressed as per cent of the anhydrous substance.*

brought to constant weight at a vapor tension of 18.1 mm. is placed in an atmosphere with a vapor tension of 3.7 mm., it loses water very rapidly and reaches a constant weight in about two days at 70.2% water, which is equivalent to $3CaO.Al_2O_3.10.52\ H_2O$. It seems therefore that a hydrate containing 10.5 moles of water is stable at vapor tensions between 3.7 and 16.1 mm., about 2.5% of adsorbed water being present at the latter vapor tension.

This hydrate is decomposed rapidly at 21° C. at aqueous tensions of 2.2 mm. or less. At 2.2 mm. the material attains constant weight in about two days with a composition of approximately $3CaO.Al_2O_3.9.5\ H_2O$. The vapor tension of this product at 21° C. appears to be in the neighborhood of 0.8 mm.

When the higher hydrates of tricalcium aluminate are placed over quicklime the water content becomes approximately that of the hydrate $3CaO.Al_2O_3.8\ H_2O$ in from four to seven days. While it is probable that there is a definite hydrate of this composition, it was found that prolonged exposure in a vacuum over freshly ignited calcium oxide continued to remove water at a very slow rate. Thus two months' exposure in a vacuum over freshly ignited lime reduced the water content of two samples of about 0.3 gram each to a composition represented by $3CaO.Al_2O_3.7.76\ H_2O$. Drying the product at 100° C., for 20 hr. reduced the water content still further to correspond to $3CaO.Al_2O_3.7.64\ H_2O$ (Table VI). The possibility of a hydrate with, say, 7.5 moles of water, is not excluded; but it is more likely that there is a hydrate $3CaO.Al_2O_3.8\ H_2O$ with a vapor tension very nearly the same as that of calcium hydroxide.

Density of the Hydrates.

Density determinations were made on only two of the hydrates described above. The results are given in Table VII.

TABLE VII

DETERMINATIONS OF DENSITIES OF HYDRATES AT 20.0° C.

Exp. No.	$3CaO.Al_2O_3$ Sample No.	Composition of hydrate	Weight of hydrate in grams	Kerosene displaced* in grams	Density of hydrate in grams per cc.
1	1	$3CaO.Al_2O_3.8H_2O$	1.3281	0.5048	2.130
2	3	$3CaO.Al_2O_3.8H_2O$	1.0648	0.4045	2.131
Mean		$3CaO.Al_2O_3.8H_2O$			2.13
3	3	$3CaO.Al_2O_3.10.5H_2O$	1.0420	0.4139	2.038

**Density of Kerosene at 20° C. = 0.8097 gm. per cc.*

If on the basis of the formulae $3CaO.Al_2O_3.8H_2O$ and $3CaO.Al_2O_3.10.5H_2O$ the molal volumes of the two materials be calculated from the density values recorded in Table VII, the figures 194.5 and 225.4 cc., respectively, are obtained. The difference between the molal volumes is 30.9 cc. and the volume occupied by 2.5 moles of water at 20° C is 45.1 cc. It appears then that when $2.5H_2O$ is added to $3CaO.Al_2O_3.8H_2O$ to form $3CaO.Al_2O_3.10.5H_2O$, a contraction of 14.2 cc. takes place in the molal volume.

The Heats of Solution of the Hydrates of Tricalcium Aluminate in HCl.200 H_2O[1]

Calcium hydroxide, prepared by exposing calcium oxide to water vapor at room temperature, readily adsorbs large quantities of water vapor with a very slight change in the molal heat of solution of the material in HCl.200 H_2O (4). Thus the heat of solution of calcium hydroxide containing 0.4% excess water was found to be only three calories higher per gram CaO than that of similar material containing 13% excess water. The differences in the heats of solution of the products obtained by the gradual drying of the hexagonal form of hydrated tricalcium aluminate, as described in Table V, might therefore be expected to furnish evidence for or against the existence of the several different hydrates. Determinations of the heats of solution of hydrated materials, corresponding to $6H_2O$, $8H_2O$, $10.5H_2O$, and $12H_2O$ in HCl.200 H_2O were therefore made.

The heat of solution of the hydrate of the composition $3CaO.Al_2O_3.6H_2O$ at 20° C. was found to be 519 calories per gram anhydrous tricalcium aluminate. The heats of solution of the other hydration products were, respectively, 21, 37 and 47 calories per gram lower. The differences are not very large, but are in excess of what one would expect to be caused by adsorption, and support the view that a series of hydrates exists.

The Optical Properties of the Hydrates of Tricalcium Aluminate.

The published data on the optical properties of hydrated tricalcium aluminate are conflicting. The values for the refractive indices vary considerably; in most cases there is given neither the analysis of the sample used, nor an exact description of the treatment to which the material was subjected prior to the determination of the refractive index. The work described in the present paper indicates that the variations reported in the refractive index may have been due to variable content of water. Table VIII gives a summary of some of the data already published and Table IX the results obtained by the authors for materials of known composition.

TABLE VIII

OPTICAL DATA ON HYDRATED TRICALCIUM ALUMINATE

Authors	Composition	Description	Refractive indices		Optical character
			ω	ε	
Klein and Phillips (2)	$3CaO.Al_2O_3.XH_2O$	Hexagonal plates, needles and spherulites	1.535 ± 0.003	1.552 ± 0.003	Uniaxial positive
Klein and Phillips (correction in paper by L. S. Wells) (6)	$3CaO.Al_2O_3.XH_2O$	Hexagonal plates, needles and spherulites	1.520 ± 0.003	1.504 ± 0.003	Uniaxial negative
Pulfrich and Linck (3)	$3CaO.Al_2O_3.7H_2O$	Hexagonal plates	1.538 ± 0.0015	1.523 ± 0.0015	Uniaxial negative
L. S. Wells (6)	$3CaO.Al_2O_3.XH_2O$	Hexagonal plates, needles and spherulites	1.535 ± 0.004	1.515 ± 0.005	Uniaxial negative

[1] *The calorimetric measurements of the heats of solution of the hydrated materials were made by Weldon G. Brown.*

TABLE IX

DETERMINATIONS OF OPTICAL DATA ON HYDRATED TRICALCIUM ALUMINATE

Method of preparation	Composition	Description	Refractive indices (sodium light)		Optical character
Hydrated below 21°C., then dried to constant weight over:			ω	ε	
$Pb(NO_3)_2+H_2O$ (saturated)	$3CaO.Al_2O_3.12H_2O$	Aggregates of hexagonal plates	1.527±0.002	1.505±0.003	Uniaxial negative
$KCl+H_2O$ (saturated)	$3CaO.Al_2O_3.10.5H_2O$	Aggregates of hexagonal plates	1.530±0.002	1.510±0.003	Uniaxial negative
CaO	$3CaO.Al_2O_3.8H_2O$	Aggregates of hexagonal plates	1.538±0.002	1.520±0.003	Uniaxial negative
Hydrated in steam at 150°C. and dried to constant weight over CaO	$3CaO.Al_2O_3.6H_2O$	Rounded grains and trapezohedrons.	N=1.604±0.002		

When hydrated tricalcium aluminate composed entirely of hexagonal plates is gradually dehydrated, to the composition $3CaO.Al_2O_3.8H_2O$, the data in Table IX indicate that the material persists as uniaxial hexagonal plates, or aggregates of such plates, with no indication of a change in the crystal form or habit. The only difference noted was a rougher appearance of the aggregates and plates of the final material. Thus, when tricalcium aluminate is hydrated at temperatures of 21° C., or below, and then dehydrated, there is no definite optical evidence for the formation of separate, distinct, crystalline hydrates.

X-ray Diffraction Pattern of the Hexagonal Form of Hydrated Tricalcium Aluminate.

As stated above, it was found that on dehydration of hexagonal plates of hydrated tricalcium aluminate at 21° C., no change was observed in the shape of the crystals. The change in the refractive index during progressive dehydration is very gradual, and the change produced in the heat of solution is not large. It is interesting to note further that the X-ray diffraction patterns for the $8H_2O$, the $10.5H_2O$ and the $12H_2O$ hydrates were almost identical. The results obtained from the X-ray pattern are given in Table X.

TABLE X

DATA FROM POWDER PHOTOGRAPH OF THE HEXAGONAL FORM OF HYDRATED TRICALCIUM ALUMINATE

d_{hkl} in Å	Estimated intensity	Miller indices	Relative spacing	Lattice constant a_o in Å
2.86	S	110(1)	.5000	5.720
2.70	W	102(1)	.4732	5.706
2.47	SS	100(2)	.4330	5.705
2.31	W	201(1)	.4043	5.713
2.14	W	112(1)	.3744	5.715
1.970	WW	103(1)	.3454	5.703
1.865	M	120(1)	.3273	5.698
1.655	SS	100(3)	.2887	5.733
1.622	WW	203(1)	.2842	5.707
1.532	WW	104(1)	.2686	5.704
1.430	M	110(2)	.2500	5.720
1.370	W	130(1)	.2402	5.704
1.080	M	140(1)	.1890	5.714
			Mean $a_0 = 5.711$ Å ± 0.02	

The hexagonal form of hydrated tricalcium aluminate was found to have a closely-packed hexagonal crystal lattice with a unit cell having the following dimensions:

a_o (side of equilateral triangle) = 5.711 Å

c_o (height of prism) = 6.453 Å

C or c_o/a_o (axial ratio) = 1.13

On the basis of the density given in Table VII for $3CaO.Al_2O_3.8H_2O$ it appears that there are 1.5 molecules in the unit cell. If the height c_o of the prism is doubled, in order that the unit cell may contain an integral number of molecules, the axial ratio becomes 2.26. The density calculated on this basis for $3CaO.Al_2O_3.8H_2O$ is 2.07; that determined experimentally 2.13. Further experimental work is in progress to check these results and to determine what effect, if any, the molecules of water have on the crystal structure of the higher hydrates.

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THE DISCOLORATION OF HALIBUT[1]

BY F. C. HARRISON[2]

Abstract

The greenish-yellow discoloration of the ventral side of *Hippoglossus hippoglossus* (Linn.) is described. This discoloration is often accompanied by softness of the flesh, which greatly reduces the value of the fish. The color-producing organism was isolated and identified as *Pseudomonas fluorescens*; its characteristics are given. Examination of materials from which the halibut might be infected revealed that the ultimate source of the organism was the fresh-water ice in which the fish were packed after being caught. Some sixteen other organisms, isolated from freshly caught living halibut and from sea water, are fully described.

Introduction

The halibut, *Hippoglossus hippoglossus* (Linn.) is the largest of the flat fishes. Specimens weighing over 700 lb. have been taken, although fish weighing more than 200 lb. are rarely captured in the Pacific.

The halibut is found in the Pacific, in water of depth 30-60 fathoms and of temperature 5-10°C., from Bering Strait to San Francisco and the Farallon islands. It is caught on the great rocky shelf that extends into the Pacific from the west shore of North America. In the Atlantic Ocean, halibut has in the past been closely associated with cod, but, owing to the depletion of the banks in the North Sea, European fishing boats now go to Greenland and Iceland.

The Pacific catch of halibut is an important one; the amount in pounds landed on the Pacific Coast for the years 1922-1927 was as follows:—[3]

Year	American	Canadian	Total
1922	36,623,718	12,069,500	48,693,218
1923	40,667,502	12,100,000	53,767,602
1924	45,466,056	12,225,000	57,691,056
1925	43,865,839	8,369,600	52,235,439
1926	45,252,435	9,438,900	54,691,335
1927	46,368,316	10,530,900	56,899,216

A moderate estimate of ten cents per pound shows the total value of the catch to be more than five million dollars a year; hence discoloration, which leads to loss of value, becomes of economic importance.

[1] *Manuscript received June 26, 1929.*

Contribution from the laboratories of the Pathological Institute, McGill University, Montreal. The work described was carried out under the auspices of the Biological Board of Canada.

[2] *Professor of Bacteriology, McGill University.*

[3] *Data from the "Pacific Fisherman" and U.S. Bureau of Fisheries.*

Discoloration.

In recent years fishermen and buyers have become perturbed by the increasingly large number of fish which, when landed, are discolored, or soft, or both. The discoloration is noticeable on the white or ventral side of the fish, and is greenish-yellow, sometimes more yellow than green. The color is usually in the slime that forms in considerable amount after the death of the fish, although some slime is present on both dorsal and ventral surfaces when the fish is alive. The fishermen believe that the slime acts as a preservative and are careful not to wash away any more of it than is necessary when eviscerating the fish. When slime is absent the same greenish-yellow color is manifest on the skin of the fish, and cannot be washed off. With the discoloration there is often an accompanying softness, which the grader who selects the fish before packing is quick to detect. This softening is undoubtedly the beginning of deterioration, and the next step is the beginning of putrefaction. Of course, such fish are quite unfit for sale.

Before passing on to a description of the experiments it should be noted that fish which were more or less discolored and soft were found in each and every cargo of the more than 100 fishing-boats observed at Prince Rupert. The research which will be described in this article included more than 150 examinations of fish from these boats and the securing of more than 200 pure cultures of organisms obtained from fish and other sources; the morphology and cultural characteristics of all the organisms isolated were examined, partly at the Fisheries Experiment Station, Prince Rupert, and partly in the laboratories of the Pathological Institute at McGill University.

Fishermen and buyers are all keenly interested in this discoloration, and their active interest, support and willingness to help gave great encouragement in working out the problem.

Handling of the Catch.

The methods of catching and marketing halibut are quite uniform. Larger vessels exploit the more distant grounds (voyages of 10 to 14 days are usual), while the smaller vessels obtain their catches nearer their marketing ports.

Nothing need be said of the actual technique used in catching but the methods used as soon as the fish are dumped out of the nets or thrown off the hooks will be described, as they have an important bearing on keeping quality.

The fish are taken from the deck pen and placed on a low table or hatch, the fishermen using a short gaff (or hook) in order to lift them. If not dead they are killed by a blow on the head from a stick, or club. They are then slit and the viscera are removed with a small scraper attached to a piece of hose, the cavity being washed out and all blood removed. Care is taken to leave the slime on the fish. After all have been cleaned and eviscerated they are packed in bins in the fish hold, with sufficient chopped and broken ice to fill the poke, and to allow a liberal amount between and around the fish.

The ice used is loaded in the boats just before they leave port, and, with one exception, is obtained from cold storage or ice-manufacturing plants; the one exception to this is the use of ice obtained from bergs, but this source of supply is very seldom used.

As soon as the bins are filled, the vessel is hurried to port to sell its cargo. The captain goes ashore, gives an estimate of his small, large, and medium-sized fish, names the place where he fished and the number of days he has been out. The fish are sold by auction, a larger price being obtained for the medium-sized than for the small and very large fish. After the sale the captain places his boat alongside the wharf where delivery is to be made, and unloading commences.

The fish are thrown into a stout net, hauled up on the wharf, and dumped on a large trestle table. Two of the crew stand on the table, pick up the fish with a short hook in the left hand, decapitate it with a heavy knife held in the right and throw it in front of the grader who stands at one end of the table. He seizes each fish, judges the quality, pulls out any ice in the poke, and places the fish in the scale. Other men take the fish from the scale, ice the boxes, re-ice the pokes, nail on the lids, and label them. A box usually weighs 200 lb., but the heavy fish are put into larger boxes.

While unloading is going on, other members of the crew are washing the loose boards that form the sides or ends of the bins, using fresh water and a stiff broom. All surplus ice that has been in contact with the fish is dropped overboard, and, as soon as all the fish have been landed, the hold is washed out, the bilge water pumped out, and the clean bin boards replaced.

As free access was had to all fish landed at Prince Rupert, it was very convenient to select any desired specimen for examination and to take it to the laboratory located on the unloading wharf. This was done whenever necessary.

Microscopic Examination of Discolored Fish

A great many microscopical preparations were made from the slime and skin of yellowed fish. The usual procedure was to spread a little slime on a slide, or to rub a slide against the skin of the fish, then to fix and stain by Gram's method, supplemented at times by simple staining with methylene blue or carbol-fuchsin. Gram's method, and counterstaining with safranin gave the more instructive preparations.

Under the microscope all these preparations were very similar, and little or no difference could be observed whether taken from yellowed slime and fish, or slime from less yellow fish. Neither could any difference be detected in fish coming from widely different localities, or different depths. However, fish from cargoes 10-16 days out did seem to have greater numbers of organisms, and more Gram-positive ones, when compared with preparations made from fish 6-8 days out.

Smears from slime or fish showed:

1. *Gram-positive cocci.* These occurred singly and in pairs; occasionally in tetrads, packets and small clumps. They were not numerous compared with the large numbers of organisms, but were nearly always present. These cocci were at times slightly longer than wide, probably due to stretching before division, but often cocci were present in which the surfaces in contact were slightly flattened, making the width greater than the length.

2. *Gram-positive bacilli.* Gram-positive rods were invariably present, usually in small numbers; they varied in length and thickness, some small, others of medium size with rounded ends. They usually occurred singly, never in chains.

3. *Gram-positive torulæ.* A few of small size were occasionally seen.

4. *Gram-negative cocci.* These were often quite numerous, and appeared in pairs or in short chains. Sometimes they were large, over 1μ in diameter, There was also present what appeared to be a coccoid bacillus, that is it resembled a coccus about to divide. It was difficult to state precisely whether or not in these slime preparations it was a true coccus.

5. *Gram-negative bacilli.* These constituted the greater number of micro-organisms on the slide, making up probably 90% or more. These rods were of varying size, some short and stumpy, some medium in length and diameter, others longer and more slender, with bent or distorted forms, giving the impression that there were several or numerous species present.

   Of these five classes of organisms the majority were recovered in pure culture.

Direct Infection Experiments:

A sound fish that showed no discoloration was selected, taken to the laboratory and carefully washed with absorbent cotton and sterilized water. This operation removed all the slime, and left the skin white, with the fish muscle just visible below the surface.

A little slime from a characteristic yellowed fish was then transferred with a platinum loop to the washed fish. A series of parallel streaks was thus made across the white surface of the fish, which was then covered with a clean, damp towel, so arranged that it did not touch the fish. After 18 hours in a room where the temperature was about 14°C. the fish was examined. It was found that where the fish was streaked with the slime there was a band of characteristic yellow, or greenish-yellow color, similar to that of the naturally affected fish. Between the bands the color remained white.

This simple, direct infection experiment, repeated with modifications several times, clearly demonstrated that the discoloration was due to an infection and that it would be reproduced by inoculation from an infected fish.

Isolation of the Color-Producing Organism

A special medium was made up which gave very good results, and on which the majority of organisms found in slime or on fish grew well. Five hundred grams of halibut, including bone and skin, was parboiled in 500 cc. of sea-water and 500 cc. of tap water. After filtration through a cloth, the filtrate or broth was used to make up gelatin (12%) or agar (1.5%). Potassium nitrate (0.25%) was added, and the pH adjusted to 7.0.

Gelatin plates were made in the usual way, but agar plates were usually poured, allowed to set, and then streaked either with a platinum loop or an absorbent-cotton swab, usually the latter. Large numbers of these swabs were prepared and sterilized. It was thus possible to examine a large number of specimens of slime and fish with quite as good results as if the halibut had been carried to the laboratory for examination.

Gelatin cultures were kept at room temperature (14-20°C.) and agar cultures were incubated in a thermostat at a constant temperature of 25°C.

By these methods no difficulty was encountered in isolating from gelatin or agar a green fluorescent organism, which rapidly and characteristically liquefied the former, and gave a well marked greenish-yellow zone around the agar colonies. In fact, the organism grew so fast at 25°C. that in 24 hours it had produced a green fluorescence in the whole plate and, in order to obtain discrete colonies, it was necessary either to dilute largely or to make successive streaks in a large number of plates.

The fluorescent organism was identified as *Pseudomonas fluorescens*, and will be described later with its variations. This organism has been isolated from every fish examined, and from more than 100 different cargoes.

After obtaining the organism in pure culture and from several different sources, a clean washed fish was prepared as before described, and parallel streaks were made on the clean white skin with a platinum loop charged with material from a pure culture growing on sloped agar. In 18 hours the streaked portions presented the greenish-yellow color; the remainder of the fish was white.

The following points were then established:

1. Constant association of the greenish-yellow organism *Ps. fluorescens* with the discolored halibut.
2. The isolation of *Ps. fluorescens* in pure culture, comparable to the usual description.
3. The production of the characteristic color on halibut with pure cultures from different isolations.
4. The re-isolation of the organisms from the artificially inoculated fish.
5. The growth and cultural peculiarities of the re-isolated organisms are exactly comparable to the original organisms.

Pseudomonas fluorescens (Flügge)

Over 150 different isolations of this organism have been worked out from the following sources; upland surface lake water used for drinking purposes and for the manufacture of ice; artificial ice from Butedale, Juneau, Ketchikan, Prince Rupert, San Juan, Seattle (two sources), Sitka; halibut from all localities, from Unalaska to the Hecate Strait; bilge water, bin boards, holds, landing nets, landing tables, grader's mitts, etc.

The general characteristics of the organism are as follows:—

Pseudomonas fluorescens Migula, B. fluorescens liquefaciens Flügge.

Rods, varying in size from 1 to 2 μ × 0.3 to 0.6 μ, occurring singly and in pairs. Motile with a single polar flagellum. Occasionally seen with 2 or 3 flagella. Gram-negative.

B.P. Gelatin colonies: Circular, with edges occasionally almost ciliate, liquefying, greenish in color, with irregular deposit. Growing quickly and spreading.

Gelatin stab: In 24 hours, filiaform with slight infundibuliform liquefaction at surface. As growth progresses liquefaction may be saccate and is moderately rapid. There is a greyish to pink sediment, and liquefaction is complete in from 11 to 30 days. Principal differences between isolations seem to be in the shape and speed of liquefaction.

In halibut gelatin and halibut agar there is abundant growth and greenish-yellow fluorescence which develops almost immediately. Gelatin is liquefied infundibuliform to saccate with occasional stratiform. Liquefaction complete in nine days or longer.

B.P. Agar slant: Abundant growth in 24 hours. At first whitish, with medium beneath colored a faint greenish-yellow; later the color of the growth becomes somewhat pink-red, the medium becomes markedly greenish-yellow, and may even darken later to a brownish tint. Crystals present just below surface.

B.P. Broth: Becomes very cloudy or turbid with greenish-yellow pellicle and grey sediment, with liquid between of a greenish-yellow color.

Litmus Milk: There is no coagulation but color is at first darkened to dark blue, indicating alkalinity, and there is a digestion without coagulation to a clear brownish liquid with dark blue on the surface. This takes place in from 7 to 30 days.

Potato: Abundant growth, spreading and becoming in course of 5 days or longer a light cinnamon-pink to sepia-brown color. Various cultures show a good deal of difference in the shades of colors formed, some remaining light, and others becoming quite dark.

Nitrate Broth: Nitrates are reduced to nitrites. Ammonia and nitrogen are also formed in this broth.

Lead Acetate Agar: Good growth, which often changes to a yellowish-brown color, but there is no darkening of the medium.

Glucose Broth: Becomes cloudy, and acid generally forms, but no gas.

No acid or gas in other sugars when kept for 40 days.

Loeffler's Blood Serum: Brownish, shiny, spreading growth which becomes slightly viscous; in most cases there is slight liquefaction of the serum.

Optimum temperature about 25° C. Does not grow at 37° C. At temperatures of 2 to 4° C. there is good growth in 4 days.

Comparing this description with those in the manuals, one finds minor differences. A single polar flagellum is noted by Bergey. Chester describes a bundle of three to six polar flagella. Bergey makes no mention of acid in glucose broth. There are great differences in the rapidity and manner of liquefaction and in the color on potato. The low temperature at which the organism can grow is also significant in its practical aspects.

Relation of Ice to Discoloration.

As *Ps. fluorescens* is an aquatic organism, frequently present in the water of rivers, streams, lakes and ponds, and is particularly abundant in waters containing much vegetable debris, numerous examinations were made of water and of ice manufactured from these upland surface waters. In no case had the water been filtered or chlorinated.

The freezing process does not destroy this organism, which remains alive in the ice but is unevenly distributed. In the experiments it has been observed that artificial ice contains, as a rule, more organisms than good natural ice.

Artificial ice is made by first pouring water into rectangular iron containers, which are immersed in brine. The water begins to freeze where it is in contact with the tank, and hence any vegetable matter or debris is forced by this freezing action to the centre. The unfrozen core is pumped out to eliminate this material, fresh water is added, and the process continued until the whole mass is frozen. The tanks are then hauled out of the brine, dipped for a few minutes into hot water, and the ice block, weighing about 400 lb., turned out. Thus the bacteria in the water are frozen into the ice, and are unevenly distributed in it. On the other hand, although the green organism may be found in natural ice, the process of freezing from the surface downwards appears to eliminate the bacteria to a large extent.

Many samples of ice from refrigerating plants, or from the boats, have been examined. In the laboratory, a clear piece was selected, well washed in sterilized water, and then thoroughly flamed by holding it in sterilized forceps, and playing the flame of a Bunsen burner all around it until it was reduced to the size of a walnut. The piece was then transferred to a sterile dish, or wide-mouthed flask; after being allowed to melt at room temperature, the water was plated out in the usual way, using halibut agar or gelatin. The wide variation in the total numbers of colonies and of *Ps. fluorescens* in water from different sources, is shown in Table I.

TABLE I

PREVALENCE OF *Ps. Fluorescens* IN WATER FROM VARIOUS SOURCES

Source, identification and remarks	Total No. of colonies per cc.	*Ps. fluorescens* per cc.
Butedale ice, Ex. G. (name of halibut boat). The number of green liquefiers was in reality larger, as the plates had to be counted early.	26,000	2,200
	24,000	1,800
	22,000	3,200
	26,000	3,500
Butedale ice. Ex. S.	320	120
	320	120
	320	80
	280	80
Juneau ice. Sample 1.	640,000	80,000
	800,000	90,000
	760,000	70,000
Juneau ice. Sample 2.	60,000	2,000
	53,000	1,500
	50,000	1,800
Ketchikan ice. Sample 1.	44,000	11,000
	51,000	9,000
	96,000	11,000
	87,000	13,000
Ketchikan ice. Sample 2. (from a block)	48	16
	48	32
	200	50
	80	20
Ketchikan ice. Sample 3.	23,000	8,000
	49,000	7,500
	36,000	8,000
Ketchikan ice. Sample 4. Average of three plates.	103,000	20,000 liquefiers
Ketchikan ice. Sample 5.	7,000	800
	6,500	600
	6,200	600
Petersburg ice. Ex. A. Average of 4 plates.	45,200	22,000
Petersburg ice. Ex. B. Average of 4 plates.	3,840	560
Petersburg ice. Sample B. Average of 4 plates.	1,900	800
		No. of green liquefiers
Prince Rupert. Water from tap after running 4 hours.	180	90
	120	80
	140	85
	160	80
Prince Rupert. Ice direct from outside of block 15 min. after being drawn. Average of 6 plates.	16	5
Prince Rupert ice, crystal clear, near centre of block. Average of 4 plates.	7	4
Prince Rupert ice, near centre core.		*Ps. fluorescens*
Average of 4 plates.	400	52
Prince Rupert ice, discolored portion. Average of 4 plates.	1,540	1,200
*Prince Rupert ice, sediment. Average of 4 plates.	25,000	5,000
San Juan ice. Sample 1.	204	96
	1,280	160
	380	120
San Juan ice. Sample 2.	1,440	540
	1,600	320
	1,400	440
	1,600	320
Seattle ice. Sample 1.	1,200	240
	1,200	260
	900	160
	1,000	160
	700	240
Seattle ice. Sample 2.	350	150
	400	150
	600	300
Sitka ice. Sample 1.	13,200	1,700
	1,000	900
	1,200	600
Sitka ice. Sample 2.	9,000	3,000
	13,000	3,000
	49,000	12,000
Natural ice from some distance inland, stored for 6 months before use.	20	0
	22	0
	22	0

**A number of other examinations of Prince Rupert ice were made, but those given above are typical of the wide variety in numbers and fluorescent content that was obtained.*

When ice was obtained which had been on board the fishing boats for 7 to 15 days—sometimes stored in the hold, and at other times after being in contact with fish—great care was exercised in the selection of clear pieces, in cleaning and washing the outer portion, and in heating until only a small piece remained for examination. Yet it may be noted that such ice nearly always gave higher counts than ice obtained from a large block out of the warehouse. The uneven distribution of organisms in a 400-lb. block is evidenced by the Prince Rupert results, which show that the organisms are forced inward, and are found in largest numbers in the core of the block, which is the part last frozen. The relatively small number of bacteria in natural ice which has been stored some time is noteworthy.

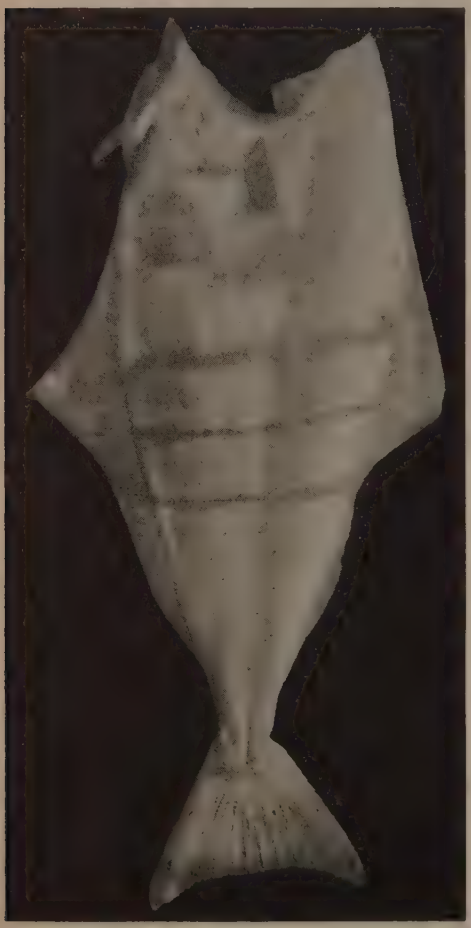

FIG. 1. *Inoculation of Halibut with the Green Fluorescent Organism from Five Different Ice Supplies. The bands of color were greenish-yellow in the original.*

Reproduction of Color with Ps. fluorescens Obtained from Various Ice Supplies.

The belly of a clean, fresh, medium-sized halibut was swabbed with absorbent cotton moistened with sterilized water. Five different cultures of *Ps. fluorescens*, 24 hours old and isolated on halibut agar from five different ice supplies, were streaked in parallel lines across the white side of the fish, which was then covered and allowed to remain at room temperature (15° C.) for about 18 hours. At the end of this time there were five distinct bands (Fig. 1) of greenish-yellow color where the organism had grown and spread on the skin. *Ps. fluorescens* was recovered from these bands.

Examination of Bilge Water, Bin Boards, Landing Net, Floor of the Hold, Trestle Table, and Mitts Worn by the Grader.

Rubbings were secured from all the above-mentioned sources by means of swabs of cotton sterilized in test-tubes, or else actual material was obtained in sterilized containers and taken to the laboratory. From the evidence secured it may be said that everything which comes in contact with ice, or water from melted ice, especially after it has been in contact with fish from which bacterial nutriment is obtained, is very heavily infected, not only with *Ps. fluorescens* but with a large number of other organisms which materially affect the keeping quality of the fish. A typical group of results is presented in Table II. The average of four plates of halibut gelatin is shown in each case.

TABLE II

BACTERIOLOGICAL EXAMINATION OF BILGE WATER*

Source	No. of colonies per cc.	Liquefiers per cc., mostly *Ps. fluorescens*
Ex. Halibut boat P.	165,000,000	25,000,000
Ex. Halibut boat I.	172,000,000	32,000,000
Ex. Halibut boat L.	300,000,000	12,000,000
Ex. Halibut boat M.	160,000,000	15,000,000

**Bilge water seeps through the bins into the hold. It consists of the drainage from the melting ice in which the fish are packed, together with a certain amount of sea water.*

Slime and the Penetration of Organisms.

It is believed by fishermen that the slime or mucus on the surface of the halibut is an important factor in preservation of the fish. They are careful not to remove the slime when washing the fish after evisceration, although more slime forms when the fish are dead, providing they are not frozen. Slime is, however, lacking on some fish for various reasons, and the discoloration is then directly on the skin; these fish are often soft and are rejected by the grader. The explanation is that the slime protects the skin, and, while it affords excellent food for micro-organisms, some considerable time is required, at the low temperatures employed, for the bacteria to grow through and attack the underlying skin.

Slime was collected from a number of fish and analysed by Dr. Ingvaldsen of the Prince Rupert Fisheries Station. In three cc. of slime 33.348 mg. of nitrogen was found, and of this 11.9% or 3.9744 mg. was amino nitrogen. No free carbonhydrate was present, but on hydrolysis some was found. This slime, therefore, so rich in nitrogen, forms excellent food for the bacteria, while the sea water provides mineral matter and other salts.

Bacteria grow well on the surface of the skin, and, as many are proteolytic, they seem to be able to penetrate the flesh rather quickly. To what extent the general bacterial infection takes place through the gills and by way of the poke, as compared with skin infection, cannot be stated, as facilities did not permit us to examine this point. The author has, however, investigated

the bacterial content of fresh haddock, and has found that the flesh is sterile, when examined immediately, but that infection by way of the gills and the poke takes place quickly after death. Halibut that is not quite fresh certainly shows the first evidence of going bad at the poke, but since many fish are soft and unmarketable even when they are landed it is considered that a certain amount of infection takes place through the skin. The following experiment illustrates this point.

A yellowish fish was taken to the laboratory, and a large piece of flesh with adherent yellowed skin was removed, all necessary precautions to prevent infection being taken. Cultures were made from the outer surface of the skin. This piece of skin was then removed with a pair of sterilized forceps, and cultures were made from the inner surface and the flesh attached to it. Small pieces of flesh were then removed at varying depths, by touching the flesh with a hot needle. In this way small portions of flesh were taken at different depths. These pieces were then transferred to culture media. After triturating in halibut broth, gelatin, and agar, plates were poured, with the following results:

1. *Outer surface of the skin* — Very large numbers of *Ps. fluorescens*, a white, moist, shiny colony and a few chromogens.

2. *Inner surface of the skin.* From three different areas large numbers of *Ps. fluorescens*, and a white, moist, shiny colony.

3. *Flesh at a depth of 1 cm. from skin.* Large numbers of *Ps. fluorescens*, and a white colony.

4. *Flesh at a depth of 2 cm. from skin.* *Ps. fluorescens*, and a large number of the same white colony.

This experiment seems to prove that penetration of the organism does take place and that it induces proteolytic changes in the flesh of the halibut.

Examination of Fresh Halibut

Since some doubt existed as to the ultimate source of infection of the fish by *Ps. fluorescens*, it was decided to examine the fish before they came into contact with the boat, and also the sea-water in which they were caught. Through the kindness of Capt. Chris. Hendricksen of the halibut boat "Gibson" the author and Professor Sadler were enabled to make a short voyage and to obtain a number of halibut of various sizes from a bank in latitude 54° 19′ and longitude 130° 37′. A number of skates of gear baited with herring were dropped, and, after two hours, were hauled in by the gurdy. As the fish came to the surface, the gurdy was stopped and a halibut was gaffed through the head. Before it touched bulwark, deck, or anything else, the ventral side was rubbed hard with a sterilized absorbent-cotton swab on a stiff wire, which was immediately placed in a sterilized test tube. Some two dozen halibut of various sizes were swabbed in this manner, and each swab was placed in a separate tube. These tubes were then taken to the laboratory, and cultured on sloped halibut agar, or agar plates; after incubation for several days at 25°C. they were carefully examined for the presence of

Ps. fluorescens. In no instance was this organism found, but a collection of other bacteria which were on the surface of the fish was observed. Undoubtedly, after the fish is dead and packed in ice, these latter increase in the slime and give rise to products which will cause deterioration and interfere with keeping quality. The significant fact, however, is the absence of *Ps. fluorescens* on fish freshly caught. It is therefore clear that ice, or the water from which the ice is derived, is the only primary source of this organism that has been found.

Samples of sea-water from a depth of 30 fathoms were also taken and examined culturally. This water was found to contain bacteria, but no *Ps. fluorescens*.

The organisms from the freshly-caught halibut and sea-water were isolated from sloped halibut agar, repeated several times, and re-isolated and cultured. They proved to be a very interesting collection, some of them being identical or almost identical with well-known bacteria; others have not been identified with any described organism in the literature accessible. The known forms have not previously been described as existing on the ventral surface of living halibut, or in the water, at depths of 30—60 fathoms in the Pacific Ocean. These isolations have shown conclusively the origin of many of the organisms which had been noted in the slime, and on the skin of fish landed at Prince Rupert.

An examination of freshly-caught fish similar to that described above was made by Capt. N. Pedersen, of the "Morricet", in Hecate Strait and Dixon Entrance. The swabs were cultured and examined at Prince Rupert. The results showed a collection of bacteria similar to those on fish caught by the "Gibson", with *Ps. fluorescens* again conspicuously absent.

Description of Organisms Isolated Directly from Freshly Caught Living Halibut and from Sea Water

In Tables III, IV, and V and on the following pages appear detailed descriptions of sixteen of the organisms mentioned above. With reference to the cultures, the media used were made, unless otherwise stated, according to the methods given in the Manual of Methods of the Society of American Bacteriologists.

The proteose medium was made with 2% proteose (Difco) and cultures were made on this, also in other tubes containing in addition either 2% dextrin, or 2% gum arabic. Some forms browned the latter two media. Ammonia phosphate broth and agar, with dextrose, had brom-cresol-purple as indicator. The morphology described is from cultures at room temperature, 24 hours old; at 37°C. and 2°C. cultures were five to seven days old. The colors on various media were compared with Ridgway's Color Charts (6), and his nomenclature used in describing them.

Anaërobic cultures were made by exhausting the air in a jar, and at the same operation spilling the soda solution on pyrogallic acid to absorb the remaining oxygen.

TABLE III

	Micrococcus varians Migula (5)	*Micrococcus citreus* Migula (5) or *Staphylococcus citreus* Bergey (2)	*Micrococcus flavus* Lehmann and Neumann (4)	*Micrococcus aurantiacus* (Schröeter) Cohn (7)	*Micrococcus candidus* Cohn
Description	Spheres 1μ when actively growing stretches somewhat longer than 1μ. At 2° occurs singly in pairs and small clumps,—smaller, less than 1μ. At 37° uneven in size, some 1.5μ, others 0.7μ. Gram-positive. At 2°C. a number of cells Gram-negative.	Spheres averaging 0.8μ. At 2° C. occurs singly and in pairs, average diameter 1 μ. At 37° more irregular in size from 0.5 to 1.0 μ, cells in pairs, often very uneven in size. Occasional groups of three cells, the three of uneven size. Gram-positive, some cells Gram-negative.	Spheres from 0.6 to 0.8 μ occurring in pairs and tetrads, often short chains of three or four; surfaces in contact flattened. Gram-positive, some cells negative. At 2° C. coccus single and in pairs, small clumps av. 1.0 μ. uneven in size, one large and a much smaller cell, often together. Same at 37°. Non-motile.	Spheres average 1μ occurring singly, in clumps and short chains. Gram-positive. At 2° C. cells less than 1μ often Gram-negative; at 37° tendency to form clumps, often in tetrads. Gram stain: cells, centre violet, margin pink.	Spheres 1.0 to 1.3μ. Occurs singly and in pairs and occasional short chains of three to four. Surrounded by capsule. (Gins and Hiss method). Gram-negative.
Gelatin colonies	Punctiform, opaque, rough edge, capitate, whitish-yellow, moruloid.	1 to 2 mm. in diameter, with a white centre spot 0.5 mm. in diameter, pale yellow, liquefying 6 days. Edge entire, granular.	Punctiform, yellow, edge entire, shiny granular.	Bright yellow, punctiform, pulvinate, circular, entire edge, granular.	Up to 2 mm. in diameter, white, iridescent, convex to pulvinate, shiny, smooth, edge entire, granular.
Gelatin stab.	Slightly raised yellow surface growth, filiform below. No liquefaction (43 days).	Filiform, slightly yellow near surface, complete liquefaction in 43 days, cloudy liquefied gelatin at surface, clear below.	Pale yellow, slight surface growth. Edge raised in older cultures, upper part of filiform growth, yellow. Beginning to pit in five days. Slight liquefaction in 22 days. Liquefaction in 43 days.	Surface growth raised, shiny, slight spreading, pale yellow. Filiform streak yellow. No liquefaction (33 days).	Slight, white, raised surface growth, iridescent, filiform below. No liquefaction (24 days).
Agar colonies	Minute to punctiform.	From 1 to 1.5 mm. Pale yellow, shiny, pulvinate, smooth, edge entire, granular; colonies, if touched with a needle, very elastic.	Punctiform, 1 mm. bright lemon-yellow, capitate, smooth, shiny, edge entire, granular, elastic when touched with needle.	1 to 2 mm. in diameter, bright yellow, circular, pulvinate, shiny, smooth, edge slightly rough.	Punctiform, 1 mm. in diameter, whitish, shiny, smooth, edge entire, granular, iridescent by reflected light.
Agar slant	Martius-yellow, smooth, shiny, slightly raised and spreading, moderate growth.	Martius-yellow, shiny, smooth, spreading, slightly raised, moderate growth. Elastic if touched with needle.	Martius-yellow, shiny, smooth, spreading, moderate growth.	Buff to light orange-yellow, slightly raised, shiny, smooth, spreading, moderate growth.	White, shiny, raised, very iridescent, rapid growth.

Broth	Cloudy, followed by clearing with yellow sediment.	Cloudy at first, then clearing 22 to 30 days; a pellicle forms and yellow sediment.	Cloudy and yellow sediment.	Turbid and flocculent with film ring and sediment clearing in 54 days with pellicle and sediment.	A pellicle forms, broth cloudy, with sediment.
Purple milk	Faintly acid. No coagulation or other change.	Bright yellow broad ring, yellow sediment; no other change.	Faintly acid, no coagulation or other change, yellow sediment.	Faintly acid, no coagulation, yellow sediment.	Slightly acid, but no coagulation. (Litmus Milk).
Potato	Scant, shiny, Martius-yellow, and transparent growth.	Martius-yellow, first rather transparent becoming opaque, moderate, smooth, shiny growth.	Scant, shiny, semi-transparent, light yellow growth.	Moderate, spreading, shiny, light orange-yellow growth.	Glistening, restricted growth.
L. blood serum	Martius-yellow, shiny, smooth-spreading moderate growth.	Martius-yellow, spreading, shiny, smooth, semi-transparent growth.	Lemon-yellow, spreading, shiny, smooth, moderate growth.	Deep chrome-yellow, smooth, shiny, slightly spreading moderate growth.	White, shiny, semi-transparent, raised, smooth, abundant growth.
Dextrose	Acid	Acid	No acid	Acid	No acid
Lactose	Acid	Acid	No acid	No acid	No acid
Saccharose	Acid	Acid	Faintly acid	Acid	No acid
Salicin	No acid	Acid	—	No acid	—
Raffinose	Acid	Acid	—	No acid	—
Mannite	Acid	Acid	No acid	Acid	—
Inulin	No acid	Acid	—	No acid	—
Glycerine	Acid	Acid	Acid	—	—
Levulose	—	—	—	Acid	
$NH_4H_2PO_4$ broth	Clear	Clear	Clear	Clear	—
$NH_4H_2PO_4$ agar	Scant growth (13 days)	Scant to slight growth (13 days).	No growth to scant growth (13 days).	Scant growth, 13 days.	Clear, at first cloudy (13 days).
Pb. Ac. agar	Shiny, whitish, semi-transparent growth.	Whitish spreading, slight growth, medium unchanged.	Brown, shiny, moderate growth, media unchanged.	Light orange-yellow growth.	Growth in six days, moderate growth 13 days.
Proteose 2% media.	Martius-yellow, shiny, smooth, abundant, slightly raised growth.	Martius-yellow, shiny, smooth, slightly raised, moderate growth.	Lemon-yellow, moderate, shiny, smooth, slightly raised growth.	Pale orange-yellow, spreading, shiny, moderate growth.	Brownish growth, shiny. No change in medium.

TABLE III—*Continued*

	Micrococcus varians Migula (5)	*Micrococcus citreus* Migula (5) or *Staphylococcus citreus* Bergey (2)	*Micrococcus flavus* Lehmann and Neumann (4)	*Micrococcus aurantiacus* (Schröeter) Cohn (7)	*Micrococcus candidus* Cohn
Halibut gelatin	Pale yellow surface growth, slightly raised. Filiform white. No liquefaction in 14 days.	Surface growth Martius-yellow, slightly raised, filiform, pitting in 8 days.	Slight yellow surface growth, filiform, no liquefaction (14 days).		—
Indol	Not formed.	Not formed	Not formed	Not formed	
Nitrate broth	Reduced to nitrites (strong), trace of ammonia.	Trace of nitrite, trace of ammonia.	Trace not reduced, trace of ammonia.	Trace of nitrite, trace of ammonia.	—
Temperature	Growth at 37° slight; at 2° slight; good growth at 20 to 25° C.	At 37° very slight growth; at 2° slight growth; at 20 to 25° good growth.	At 37° good growth; at 20 to 25° good growth; at 0 to 2° C. very slight growth.	At 37°, growth; at 25°, good growth; 0° to 2° slight growth.	Trace of nitrite, trace of ammonia.
Pathogenicity	For mice and guinea pigs subcutaneous inoculation of half the growth on an agar slant—negative.	Subcutaneous in mice and g. pigs of half the growth on an agar slant. No results.	—		—
Habitat	Ventral surface of living halibut from 40 fathoms, Pacific Ocean.	Isolated from living halibut obtained at 40 fathoms, Pacific Ocean.	Isolated from ventral surface of living halibut obtained at 40 fathoms, Pacific Ocean.	Isolated from ventral surface of living halibut obtained at 40 fathoms, Pacific Ocean.	Isolated from ventral surface of living halibut at 30 to 40 fathoms, Pacific Ocean.
General	This organism resembles *Micrococcus varians* and agrees generally with Hucker's analysis (3) of 13 strains of *M. varians*.	This organism is peculiar in its sugar fermentations, fermenting all the test sugars. It does not grow well at 37° C. Hucker, who studied 29 strains of this organism, states that it is found primarily on the human body.	This organism differs somewhat from descriptions. Hucker (3) studied 26 strains, 20 of which utilised ammonia nitrogen. The slow liquefaction of this strain is noticeable. No motility in any of above isolations.	This organism resembles *aurantiacus* (Schröeter) (7) except that growth on potato is not shiny, agar colony with rough edge.	This organism is Gram-negative, but in some of its cultural features resembles *M. candidus*. Cohn. Hucker in his analysis of 13 strains states that he has never encountered a strain with just the same characteristics.

TABLE IV

	Sarcina lutea Schröeter (7)	*Rhodococcus agilis* (Ali Cohen) Holland (1)	*Flavobacterium fucatum* Harrison N.Sp.	*Flavobacterium turcosum* (Zimmerman) Bergey et al (2)	*Flavobacterium maris* Harrison N. Sp.
Description	Spheres 1 μ in pairs, fours and packet-shaped masses of eights and more. At 2° C. packet-shaped masses, surfaces in contact flattened, tetrads, eights and larger. At 37° C. similar. Gram-positive, easily over-stained.	Spheres, occurs singly, in pairs and tetrads and short chains of 3 or 4 cells. Surfaces flattened when in contact. Average size 1 μ. At 2° C. it occurs singly, and in pairs and small clumps. Average size 1 μ. At 37° C. somewhat uneven in size, a large and a much smaller cell when in pairs. From less than 1 μ to 1.25 μ, unevenly stained. Gram-positive, a few cells Gram-negative at room temperature. Motile, one flagellum (Casares-Gil's method).	Rods 2.5 to 3.5. by 0.8 to 1 μ, some slightly bent with rounded ends. At 2° C. medium sized bacillus 2.5 by 0.7 μ rounded ends. At 37° C. smaller than at 2° C. beaded, bent and uneven staining, granules, diptheroid forms. Non-motile. Gram-negative.	Rods 1.3 by 0.4 μ, often in pairs. At 2° C. small bacillus 1.5 to 2.0 by 0.5 μ rounded ends, cells often uneven in diameter, one end thicker than the other. At 37° C. very uneven in size and shape, some long and then 3 to 4 μ, others coccoid and less than 1 μ, beaded, short chains with pseudo-branching and drumstick shapes. Uneven in staining. Motile, monotrichous. Gram-negative from cultures at 20° C. Gram-positive and negative from cultures at 37° C. and at 2° C.	Rods 1.0 to 1.2 by 0.7 to 0.8 μ singly and in pairs. At 37° C. coccoid, capsulated average 1 μ. Same at 2° C. Non-motile. Gram-positive.
Gelatin colonies	Punctiform 0.5 mm. yellowish, dull, waxy, pulvinate, irregular in outline, border lobate lobulate, moruloid.	0.5 to 1 mm. in diameter, pink, smooth, shiny, margin entire, granular.	Circular, yellow centre, paler at edges, up to 4 mm. in diameter, centre spot about 1 mm. In five days liquefaction commences and progresses. Edge entire, granular.	Punctiform, convex, shiny, smooth, faint yellow, edge entire, granular to moruloid, at first semi-transparent becoming opaque with age, and edge rough to lobate lobulate.	Punctiform, about 5 mm. (15 days), edge entire granular.
Gelatin stab	Yellow surface growth, 1 mm. in diameter, dull, slightly raised, rugose at edge, filiform below and white. Liquefaction started in three to eight days, at first crateriform, later stratiform, one-third liquefied in 43 days, liquefied gelatin clear, yellow deposit.	Filiform, surface growth slightly raised, pale pink, shiny, whitish below. Slight pitting of gelatin in 3 to 8 days. Crateriform. At 43 days, half liquefied, clear liquefied gelatin and pink sediment. Some strains do not liquefy gelatin.	Filiform at first, pitting begins in two days at 20°. Crateriform liquefaction, cloudy liquefied gelatin, and heavy yellow deposit, whitish pellicle. Complete liquefaction in 43 days.	Yellow, raised surface growth 1 mm. in diameter, filiform and whitish below. No liquefaction (43 days).	Red-orange raised surface growth, filiform below and yellow. No liquefaction (33 days).

TABLE IV—*Continued*

	Sarcina lutea Schröeter (7)	*Rhodococcus agilis* (Ali Cohen) Holland (1)	*Flavobacterium fucatum* Harrison N.Sp.	*Flavobacterium turcosum* (Zimmerman) Bergey et al (2)	*Flavobacterium maris* Harrison N. Sp.
Agar colonies	Colonies up to 5 mm. in diameter, yellow, umbonate, waxy, very opaque, consistency butyrous, edge rough, moruloid.	0.5 to 1 mm. capitate to pulvinate. Shiny, smooth, pale pink becoming red. Edge slightly rough and granular.	One to two mm. in diameter. Circular, buff-yellow, smooth, shiny, convex to pulvinate, edge entire, granular.	In 7 days up to 2 mm. in diameter, circular, shiny, transparent, pulvinate, edge entire, granular.	0.5 to 1 mm. in diameter, glistening, smooth, convex, orange in colour.
Agar slant	Strontium-yellow, waxy, raised and ridged, slightly spreading, abundant.	La France pink, shiny, smooth, spreading, moderate growth, some cultures darker in colour.	Light buff-yellow, spreading, shiny, smooth, moderate growth.	Martius-yellow, shiny, slightly raised and spreading, smooth, abundant growth.	At first orange-yellow becoming cadmium-orange and later red-orange, spreading, shiny, elastic, moderate growth.
Broth	Clear, with yellow sediment.	Slightly cloudy and clearing, pink sediment.	Cloudy becoming clear, pellicle, and yellow sediment.	Cloudy, yellow sediment.	Clear with orange pellicle and sediment.
Purple milk	Unchanged, with yellow sediment.	Faintly acid, slight pink sediment. No other changes.	Alkaline, digestion without coagulation, clear serum, yellow sediment.	Yellow sediment, no other change (43 days).	At first faintly alkaline, becoming faintly acid, orange-yellow sediment. No other change (54 days).
Potato	Strontium-yellow, spreading, waxy, slightly raised, abundant growth.	Restricted to scant, shiny, pink growth.	Pale buff-yellow, shiny, smooth, spreading, abundant, becoming orange-yellow with age (40 days).	Lemon-yellow, spreading, shiny, abundant growth.	Very scant orange growth (54 days).
L. blood serum	Strontium-yellow at first, raised, smooth, dull, spreading, followed by almost complete liquefaction in 43 days.	Geranium pink, spreading, shiny, smooth, abundant. In 22 days liquefaction and pink sediment. Grows best on this medium.	Light buff-yellow, spreading, shiny, smooth, moderate growth. Colour becomes ochraceous salmon with age. No liquefaction.	Martius-yellow, smooth, spreading, moist, becoming duller with age.	Cadmium-orange, shiny, very abundant yellow under condensation-water. Best growth on this medium.
Dextrose	Faintly acid	Acid	No acid	Acid	Faintly acid
Lactose	No acid	Acid	No acid	No acid	No acid
Saccharose	No acid	Acid	No acid	Faintly acid	No acid
Salicin	—	Acid	—	No acid	—
Raffinose	—	No acid		No acid	—

Mannite	—	Acid	—	No acid	—
Inulin	—	Acid	—	No acid	—
Glycerine	—	Acid	—	No acid	—
$NH_4H_2PO_4$ broth	Clear	In seven days clear with pink sediment.	—	—	—
$NH_4H_2PO_4$ agar	Good growth in six days, and also in 13 days.	Slight growth 13 days.	Slight to moderate growth.	Slight growth (12 days).	Good growth, orange, slight acidity.
Pb. Ac. agar	Deep dirty yellow, abundant dull growth, no change in medium.	Dirty pink, shiny, moderate growth, no change in medium.	Growth brownish, shiny and moderate, deep dark brown in stab, medium not changed.	Dirty yellow, shiny, slightly spreading growth.	Vandyke brown, shiny, moderate growth. No change in medium.
Proteose 2% media	Strontium-yellow, waxy, raised, smooth, moderate growth.	Rose dorée, slightly raised, shiny, smooth, moderate.	Buff-yellow, raised, shiny, smooth, moderate growth.	Lemon-yellow, shiny smooth, moderate growth.	Cadmium-orange, spreading, shiny, smooth growth.
Halibut gelatin	Strontium-yellow, surface growth, raised and waxy, filiform below. In eight days slight crateriform liquefaction with yellow sediment.	Surface growth deep rose, filiform and whitish below. In nine days begins to liquefy.	Filiform at first, pitting in two days, funnel-shaped liquefaction, orange-yellow film, floccules in clear liquefied gelatin, deposit.	Yellow, shiny, surface growth, filiform below.	—
Indol	Not formed.	Not formed	Not formed	Not formed.	Not formed.
Nitrate broth	Nitrates not reduced, no ammonia.	Trace of nitrate, trace of ammonia.	Reduced to nitrites (strong). Trace of ammonia.	Not reduced, ammonia trace.	Reduced to nitrites, ammonia trace.
Temperature	At 37° abundant growth, at 2° slight growth, at 20 to 25° C. good growth.	Very slight growth at 37°, at 2° moderate growth. At 20 to 25° C. good growth.	At 37° C. very slight growth, at 2° C. very slight growth, good growth 20 to 25° C.	Slight growth at 37°, scant growth at 2°, good growth at 20° to 25° C.	Slight growth at 37° and also at 2°. Good growth 20 to 25° C.
Aërobic	—	—	Facultative. Grows best under aërobic but fair growth anaërobically.	Aërobic and fac. anaërobe. Slight growth in absence of oxygen.	Good growth. Anaërobic slight growth.

TABLE IV—*Continued*

	Sarcina lutea Schröeter (7)	*Rhodococcus agilis* (Ali Cohen) Holland (1)	*Flavobacterium fucatum* Harrison N. Sp.	*Flavobacterium turcosum* (Zimmerman) Bergey et al (2)	*Flavobacterium maris* Harrison N. Sp.
Habitat	Isolated from skin of living halibut obtained in 40 fathoms, Pacific Ocean.	Isolated from skin of halibut obtained in 40 fathoms, Pacific Ocean, and repeatedly found on skin and mucus of dead halibut landed at Prince Rupert, B.C.	Repeatedly isolated from living halibut obtained at 30 to 50 fathoms, Pacific Ocean.	Isolated from living halibut caught in 30 to 40 fathoms, Pacific Ocean, also from dead halibut at Prince Rupert, B.C.	Isolated from living halibut obtained at 30 to 50 fathoms, Pacific Ocean.
General	This organism differs from *Sarcina lutea*, Schroëter, in the following particulars: Liquefaction crateriform to stratiform; agar colonies are dull and umbonate; agar slant strontium-yellow; milk not coagulated nor alkaline; potato, abundant growth; no indol; grows well at 37° C.	—	This organism being nonmotile, liquefying gelatin, alkaline in milk, reducing nitrates, forming no indol, resembles *Flavobacterium lutescens* (Lustig) Bergey et al, (2) but differs markedly in size, appearance and color of colonies, and rate of liquefaction. This organism has been frequently isolated; other strains are very similar except that the color varies. In some media it is more orange (Mikado-orange, capucine-buff).	The growth on potato is different from Zimmerman's description, otherwise it compares generally with the original description on the media used in 1894. The above description gives fuller information.	—

TABLE V

	Flavobacterium dormitor (Wright) Bergey et al (2)	*Flavobacterium balustinum* Harrison N. Sp.	*Flavobacterium diffusum* (Frankland) Bergey et al (2)	*Flavobacterium marinum* Harrison N. Sp.
Description	Rods 1.5 to 2.0 by 0.3 to 0.4 μ, sometimes slightly bent, non-motile. At 37° very uneven in size, beaded and coccoid, swollen and odd shapes, uneven staining. At 2° C. small bacillus, uneven in size, often beaded and coccoid in form. Gram-negative.	Rods 2 to 4 μ by 0.6 μ forming small chains. At 37° very uneven in size, staining faintly. At 2° C. from 1.5 to 3.0 μ by 0.4 μ, ends slightly rounded, sometimes in small chains, bent forms. Non-motile. Gram-negative.	Rods 1.6 to 2.0 by 0.4 to 0. 5μ. Motile, peritrichous, flagella, often in pairs, rounded ends. At 37° C. in chains and shorter. Gram-negative.	Rods average 1.2 to 1.3 by 0.8 μ, short and stout, rounded ends, often in pairs, shorter individuals almost round, capsulated. Motile, peritrichous flagella usually four to five. Gram-positive and negative. Many Gram-negative rods contain blue granules. At 37° longer and thinner, capsulated, Gram-positive. At 2° C. smaller than at 37°, great variety in length, Gram-positive.
Gelatin colonies	1 to 1.5 mm. in diameter, with a central spot 0.5 mm., circular, bright yellow, edge entire, liquefaction commences in 3 days and progresses.	1 to 1.5 mm. in diameter, bright yellow centre 0.5 mm. in diameter, circular, edge entire. In 5 days liquefies with yellow spot in centre.	Up to 1 mm. circular, shiny, convex, white at first, later faint yellow, edge entire, granular.	Up to 4 mm. in diameter, at first iridescent whitish margin and pale yellow centre. Liquefaction begins in five days, edge slightly ciliate, margin hyaline and finely granular, centre opaque and coarsely granular.
Gelatin stab	Yellow transparent surface growth filiform below. Pitting commences in eight days; napiform to crateriform liquefaction follows, with clear liquefied gelatin and heavy yellow sediment. Three-quarters liquefied in 54 days.	Filiform, begins to liquefy on second day, complete liquefaction in 25 days. Yellow islet film and sediment.	Slightly yellow on surface, filiform below. Liquefaction crateriform commences in 10 days and half liquefied in 27 days.	Filiform, liquefaction begins in 24 hours, saccate to stratiform, half tube liquefied in 12 days, clear liquefied gelatin and yellow deposit. In 24 days 2/3 tube liquefied.
Agar colonies	Average 1 mm. in diameter, circular, shiny, convex, yellow, semi-transparent.	Punctiform, convex, shiny, transparent, cadmium-yellow. Tendency to run together and make a transparent, deep yellow mass.	2 to 3 mm. in diameter, white at first, changing to cinnamon-buff, shiny, smooth, convex, edge entire, margin hyaline, centre dense and granular.	1 to 2 mm. in diameter, circular, shiny, smooth, convex, pale yellow, opalescent by reflected light. Edge entire but undulate, margin hyaline, centre coarsely granular, and slight reticulate markings.

TABLE V—*Continued*

	Flavobacterium dormitor (Wright) Bergey et al (2)	*Flavobacterium balustinum* Harrison N. Sp.	*Flavobacterium diffusum* (Frankland) Bergey et al (2)	*Flavobacterium marinum* Harrison N. Sp.
Agar slant	Primuline-yellow, shiny, spreading, semi-transparent. Medium slightly yellowed (54 days).	Egg-yolk-yellow, semi-transparent, smooth, shiny, becoming brownish-yellow and duller with age. Medium slightly yellowed.	Capucine-buff, shiny, spreading.	Amber-yellow, slightly raised, spreading, shiny, transparent, smooth growth.
Broth	Cloudy then clearing with bright yellow ring and sediment.	Cloudy, yellow sediment.	Cloudy	Cloudy and sediment.
Purple milk	Faintly acid, yellow sediment, no other change.	Slightly acid, no coagulation, yellow sediment, no other change (54 days).	Unchanged (27 days).	Alkaline, digestion without coagulation. Clear serum.
Potato	Slight,transparent,yellow growth.	Scant, semi-transparent, yellow becoming darker in color.	Thin, scant yellowish growth.	Amber-yellow, becoming dirty yellow, spreading, shiny, and abundant growth.
L. blood serum	Primuline-yellow, spreading, shiny, semi-transparent, smooth, abundant. Best growth on this medium.	Confluent, egg-yolk color. Spreading, slightly transparent.	Spreading, shiny, thin, semi-transparent.	Faint yellow, moist, shiny, spreading, flat, growth.
Dextrose	Faintly acid	Faintly acid	Faintly acid (10 days)	Slightly acid (24 days)
Lactose	No acid	No acid	No acid	No acid
Saccharose	Acid	No acid	No acid	No acid
Salicin	Slightly acid	—	—	—
Raffinose	No acid	—	—	—
Mannite	Acid	—	—	—
Inulin	No acid	—	—	—
Glycerine	Acid	—	—	—
$NH_4H_2PO_4$ Agar	Slight yellow growth (12 days).	No growth or very scant (12 days).	Scant to slight (12 days).	Scant growth (12 days).
Pb. Ac. agar	Brown, shiny, slightly spreading growth. No other change.	Light brown shiny growth, no other change.	Slight yellowish growth.	Light brown-yellow streak, scant.

Proteose 2% media	Primuline-yellow, shiny, slightly spreading and raised, abundant growth.	Transparent, deep orange, shiny, abundant. Best growth on these media.	—	—
Indol	Not formed	Not formed	Not formed	Not formed
Nitrate broth	Trace of nitrite to no reduction. Ammonia trace.	Trace of nitrite. No ammonia.	Reduced to nitrite. Ammonia trace.	No reduction to nitrite. Ammonia trace.
Temperature	No growth at 37°. Slight growth at 2°. Good growth 20 to 25° C.	At 37° very slight growth. At 2° slight growth. Good growth at 20 to 25° C.	At 2° slight growth, at 37° good growth, at 20 to 25° C. good growth.	At 2° for 7 days slight growth. At 37° good growth. At 20 to 25° C. good growth.
Aërobic	Good growth. Anaërobic growth.	Growth. Anaërobic, very slight growth.	Good growth. Anaërobic, good growth.	Good growth. Anaërobic, good growth, plumose.
Habitat	Isolated from living halibut obtained 30 to 50 fathoms, Pacific Ocean. A number of isolations of this organism have been made.	Isolated from living halibut obtained at 30 to 50 fathoms, Pacific Ocean.	—	Isolated from living halibut obtained in 30 to 50 fathoms, Pacific Ocean.
General	Differs in some particulars from *Flavobact. dormitor* Wright.	—	—	This organism differs in many respects from *Flavobact. sulfaceum* Bergey et al (2).

Achromobacter pellucidum HARRISON N. SP.

Rods 1.2 to 2.0 by 0.6 μ singly, pairs and short chains, capsulated. At 2° C. 2.5 to 3.0 by 1.0 μ, rounded ends, capsulated. At 37° C. granular forms, various shapes and sizes, from coccoidal forms less than 1 μ to bacillar forms 2.0 by 0.4 μ, majority coccoid. Motile, monotrichous. Gram-negative.

Gelatin colonies. 2 to 5 mm. in diameter, circular, edge entire, granular, saucer-shaped liquefaction, with centre spot whitish and dense, most noticeable in the smaller colonies; the larger ones are more diffuse, edge of liquefied area slightly ciliate.

Gelatin stab. At first infundibuliform, becoming saccate, and complete liquefaction in 24 days. Gelatin browned at surface with pellicle. Liquefied gelatin cloudy.

Agar colonies. Circular, in five days 0.3 to 1 cm. in diameter, pulvinate, dirty white by reflected, and iridescent by transmitted light, smooth and shiny, edge entire, granular.

Agar slant. Slightly raised, moist, white and shiny growth. At 7 days growth becomes more transparent, the transparency increasing with age. At 24 days quite transparent and slimy.

Broth. A pellicle forms, clear liquid and heavy sediment. Slimy.

Milk. Alkaline, and browned, digestion without coagulation, serum browned and slimy.

Potato. Scant to no growth.

L. blood serum. Spreading, transparent, shiny, abundant growth. Medium browned and liquefied. Best growth on this medium.

Dextrose, no acid. *Lactose*, no acid. *Saccharose*, no acid.

Proteose media. Moist, shiny, whitish, spreading abundant growth, becoming more transparent with age. Best growth on this medium. On proteose dextrin, massive, tenacious growth, medium browned.

Ammonia phosphate agar. Slight transparent growth.

Indol. Not formed.

Nitrates. Not reduced. Ammonia trace.

Facultative anaërobe. Grows well under aërobic and under anaërobic conditions. At 24° C. growth is more abundant under the latter condition.

Temperatures. No growth at 37°. Good growth at 2°. Abundant growth 20-25° C.

Habitat. Repeatedly isolated from skin of halibut taken in 30-40 fathoms. Pacific Ocean.

Growth on various media. On fresh fish-agar without peptone and made with 0.5% $Ca_3(PO_4)_2$ and 3% sea salt of pH 8.0, growth was luxuriant; on agar made up from fish skin instead of flesh as above, growth was moderate; on agar made from fish scales, other ingredients as above, there was good growth; on mucous, slight growth.

All the above were tried at 24° C. and found to be aërobic and anaërobic. There was the same proportion of growth in each.

Morphology under varied conditions.

At 4° aërobic—small, slender rod; at 25° aërobic—coccoid forms; at 24° anaërobic—larger rods than at 4°.

On transfer from a transparent culture to agar, at 24° C., the coccoid form of the transparent cultures changes to long rods in 24 hours.

Reference has been made to the transparency of this organism when growing on beef-peptone agar. The organism seems to undergo autolysis, or to go through a symplastic stage. On fish-agar, at 24° C., the transparency was not apparent for 14 days when grown under aërobic or anaërobic condition. At 4° after 23 days (anaërobic) there were no signs of transparency.

The growth from a number of agar slants two days old, was washed off in normal saline and filtered through a Seitz filter. Portions of the filtrate were transferred to seven agar slants, but none showed any growth after being kept for 13 days.

A similar experiment was made with agar cultures seven days old, when the growth was quite transparent. Transfers of the filtrate to 10 agar slants were made; six showed growth, which was typical, and became slimy and transparent in 5-7 days.

This experiment was repeated, with similar results, but with a smaller number of positive cultures from the filtrate.

Aërobacter cloacæ JORDAN.

Rods, short stout bacillus 1.1 by 0.8 μ, often in pairs, capsulated. At 37° C. short, stout rods. At 2° C. granular forms. Motile. Flagella peritrichous. Gram-negative at all temperatures.

Gelatin colonies. In three days colonies up to 7 mm. in diameter, saucer-shaped liquefaction, with white central spot. Large colonies, spot more diffuse. Edge slightly ciliate, wide margin, hyaline, dense granular centre.

Gelatin stab. Infundibuliform liquefaction, complete in 7 days. Liquefied gelatin cloudy, heavy sediment and ring.

Agar colonies. Up to 2 mm. in diameter, circular, slimy, convex to pulvinate, edge entire, margin hyaline, centre opaque and granular.

Sloped agar. White, slightly raised, shiny, smooth, moderate growth.

Broth. Turbid, with pellicle, ring and sediment.

Milk. Slightly acid, soft coagulum at base, digestion with alkaline reaction, turns light brown, digestion not complete.

Potato. Whitish, transparent at first, becoming opaque and cream coloured, abundant growth.

L. blood serum. Spreading, slimy, whitish, abundant growth. Becomes brown in three days with some digestion. Best growth on this medium.

Dextrose, acid and gas, in 63 days becomes alkaline.

Lactose, acid and no gas.

Arabinose, acid and gas.

Saccharose, acid and gas, in 63 days acid disappears.

Mannitol, acid and gas.

Xylose, acid and gas.

Levulose, acid and gas.

Glycerine, acid and gas.

Raffinose, acid and gas.

Maltose, acid and gas.

Salicin, acid and gas.

Galactose, acid and gas.

Inulin, no acid.

Dextrin, acid and gas.

Sorbite, acid and gas.

Rhamnose, no acid, no gas.

Dulcite, no acid, no gas.

Broth. Moderate growth, acid reaction in three days.

Lead acetate agar. Brownish, spreading, slimy growth.

Ammonia phosphate agar. Good growth, media acid.

Indol. Not formed.

Nitrates. Reduced to nitrites.

V. P. reaction. Positive, acetyl-methyl-carbinol formed.

Proteose media. Whitish, spreading, slightly raised, abundant growth. Slight yellowing of media.

Aërobic. Facultative.

Temperatures. At 37°, good growth. At 2°, slight growth. Good growth at 20-25° C.

Habitat. Isolated from halibut obtained in 30-40 fathoms, Pacific Ocean.

This organism resembles *Aërobacter cloacæ* except that it produces acid and gas in glycerol, and no gas in lactose.

Discussion

All the cultures described were isolated from living halibut, and all of them were present on the skin of dead halibut landed at Prince Rupert, B.C. The isolation of these organisms from dead halibut was difficult, owing to the enormous number present in the slime and on the skin; the slower-growing pecies had not much chance to develop on crowded plates.

The collection of organisms is interesting in that they are in all probability primitive types, being able to grow at comparatively low temperatures, 5-8° C. That some of the cocci primarily found on or in the mammalian body have been isolated from the surface of fish is interesting and significant. *Rhodococcus agilis* is not a common organism, and is rarely found. It was, however, extremely frequent on the surface of halibut but not in large numbers, as it grows slowly at low temperatures (5 to 6°C.).

The bacilli were more difficult to classify, and the author has ventured to name those most frequently found, which do not accord with any descriptions found in the literature. The most interesting is the one named *A. pellucidum* which seems to have a symplastic stage. Certain of the chromogenic bacteria are also peculiar in the intensity of their colors. The fact that so many chromogenic organisms were present on fish at depths of 30-50 fathoms, and on the under-surface of the fish, is in itself peculiar. It is the intention of the author to obtain samples of the ocean floor, and particularly of the ledges on which halibut are found, to see if these organisms are present. From sea-water at 20-30 fathoms, no chromogens were found.

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THE CHLORINATION OF METHANE[1]

By M. C. Boswell[2] and R. R. McLaughlin[3]

Abstract

A small-scale method was first developed in which the degree of chlorination of methane to methyl chloride could be determined by analysis of the resulting gases. The optimum conditions so determined were then applied on a scale which permitted the isolation and measurement of the products. A yield of 80% or better was obtained when using as a catalyst partially-reduced cupric chloride and passing moist nitrogen, methane and chlorine in the ratios of 70:7:1 at 450° C. It was found that the proportion of chlorine could be more than doubled when 8% of hydrogen was present in the methane. Under such conditions the chlorine was completely utilized and only methyl chloride and hydrogen chloride were formed. The yield of isolated methyl chloride obtained was nearly 80% and this could be increased by operating on a larger scale. The same catalyst was successfully used in the chlorination of methane to carbon tetrachloride. A yield of 90 % was obtained, with complete utilization of the chlorine. The chlorination of ethane to ethyl chloride, with a yield of at least 75% was also shown to be possible.

Introduction

Methane is the chief constituent of natural gas. "Wet" natural gas containing higher hydrocarbons which can be profitably removed is frequently encountered in boreholes; after the removal of these valuable constituents, the residual methane often goes to waste. Such an economic loss is now taking place in the province of Alberta, Canada, where a large quantity of light oil is being recovered from wet gas with only partial utilization of the residual methane.

A number of chemical methods have been suggested for the utilization of this methane. One of these is to convert the gas into compounds for which there is an industrial demand. In seeking for such a solution of the problem, at least three possible procedures are open:

(1) The conversion of methane into higher hydrocarbons such as the constituents of gasoline. This would be practically the reverse of the ordinary "cracking" process.

(2) The fractional oxidation of methane to methyl alcohol, formaldehyde or formic acid, which are, theoretically, intermediate stages in the oxidation of methane to carbon dioxide and water.

(3) The fractional chlorination of methane to produce methyl chloride, methylene chloride, chloroform and carbon tetrachloride.

The investigation of this last method is the subject of this paper.

A good review of the literature on the chlorination of methane has been made by Jones, Allison and Meighan (4). That methane can be chlorinated to form all four products—methyl chloride, methylene chloride, chloroform

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and carbon tetrachloride—has been known for a very long time. As early as 1858 Berthelot (1) observed that mixtures of methane and chlorine reacted so vigorously as to result in the production of carbon and hydrogen chloride, unless the reaction was run very slowly. It was also found later that the chlorination of methane at a more moderate rate resulted in the production of mixtures of all four possible chlorinated products, which were difficult to separate. Moreover, the yields were not particularly good. Many investigations have been carried out for the purpose of increasing the yield of a single chlorinated product. The acceleration of the chlorination of methane by sunlight, and chlorination in the presence of catalysts have been the subject of many researches. Some of these have been very successful in the production of carbon tetrachloride, notably those of the U.S. Bureau of Mines at Washington (4) in which yields in the neighborhood of 90% were obtained, using activated charcoals and "batchite" as catalysts and ratios of chlorine to methane from 5 to 1, to 2.5 to 1.

In a further study of this problem it seemed desirable to investigate the effect of certain factors influencing the reaction of chlorine on methane and, if possible, to determine the optimum conditions for the production of a single product, methyl chloride or carbon tetrachloride. Methyl chloride was first studied.

Previous unpublished work by the authors upon the mechanism of the Deacon reaction showed that when steam is passed over cupric chloride at 450°C. a reduction occurs, resulting in the formation of what may be termed a "reduction complex", oxygen and chlorine being evolved. The properties of this complex indicated that it might serve as a good catalyst for the chlorination of methane. Subsequent experiments confirmed this opinion. The preparation of the catalyst will be described later.

It seemed that the failure of earlier investigators to obtain good yields of methyl chloride was possibly due to the use of chlorine and methane alone, and that a sharp separation of the stages of the reaction might be accomplished by the dilution of the gases with an inert gas such as nitrogen. A further reason for investigating the effect of nitrogen on this chlorination process is to be found in the fact that where chlorine and methane alone are used in the catalytic chlorination to carbon tetrachloride, the reaction must be run slowly to avoid the separation of carbon, and even explosions. Pfeifer, Mauthner and Reitlinger (7) used nitrogen for the purpose of avoiding explosions, but did not investigate the influence of the concentration of nitrogen on the yield of single products. It was hoped that useful information might be secured by a study of nitrogen concentration and of the ratio of chlorine to methane.

In general, two methods of procedure may be adopted in order to study the effect of the various factors upon the yield of a compound produced by a given chemical reaction:

(1) The yield of the compound obtained can be determined by the actual isolation and weighing of it, or (2) the yield of the compound can be calculated from data obtained in an analytical procedure which has been tested and

shown to be applicable. The latter method would not involve the isolation of the compound. According to the first method, a large amount of material must be transformed in order to minimize the experimental losses. This method is desirable in those cases where such losses are small. In cases where the losses of isolation are high the second method is preferable. Methyl chloride, with a boiling point of −23.7°C. at atmospheric pressure, obviously falls into the latter class. For this reason it was decided to work out an analytical method suited to the chlorination of methane, by which the yield of methyl chloride could be determined, and which would at the same time furnish information regarding the nature of the other reactions accompanying the formation of methyl chloride.

This investigation, then, is divided into three parts:

(1) The development of the analytical method.

(2) The study of the chlorination of methane on an analytical scale.

(3) The study of the chlorination of methane on a larger scale, as indicated by (2), with the collection and measurement of the products.

Development of the Analytical Method

There are four possible reactions in the chlorination of methane:

$$(1)\ CH_4 + Cl_2 = CH_3Cl + HCl$$
$$(2)\ CH_4 + 2Cl_2 = CH_2Cl_2 + 2HCl$$
$$(3)\ CH_4 + 3Cl_2 = CHCl_3 + 3HCl$$
$$(4)\ CH_4 + 4Cl_2 = CCl_4 + 4HCl$$

In any chlorination experiment involving the passage of definite volumes of methane and chlorine into the reaction tube, the data obtainable by a complete analytical procedure consist of : (1) the volumes of methane and chlorine taking part in the reaction; (2) the chlorine in the hydrogen chloride evolved, and (3) the chlorine in the other chlorinated products. Such data will enable one to calculate the yield of methyl chloride if that be the only product formed, or, if higher chlorinated products be simultaneously formed, to compute the minimum yield of methyl chloride. In the present experiments, the chlorine was always assumed to have formed the greatest possible amount of methylene chloride, in order to avoid the possibility of over-estimating the yield of methyl chloride. If, in reality, some chloroform or carbon tetrachloride was produced, the true yield of methyl chloride was correspondingly higher than that indicated by the experimental results given.

Much difficulty was experienced and many months of time consumed in developing a successful analytical method. The chief difficulties were: (1) the preparation of pure methyl chloride; (2) the working out of a method which would permit the determination of the free unused chlorine and the hydrogen chloride formed in the reaction but which would not interfere with the remainder of the necessary determinations; and (3) the quantitative hydrolysis of the organic chlorination products by a method which would be

without effect upon the unused methane, and which would not give rise to any products interfering with the subsequent determination of methane by combustion to carbon dioxide and water. The method of hydrolysis, moreover, must be of such a nature as to permit the hydrogen chloride formed to be accurately determined.

The Preparation of Pure Methyl Chloride.

Pure methyl chloride was required for the development of the analytical procedure. Considerable difficulty was experienced in the preparation of this gas, owing to its appreciable solubility in water (four volumes at 0°C.), its action on mercury, and the necessity for the complete exclusion of air. The following method was finally adopted:

Cold, dry hydrogen chloride was passed into a flask containing anhydrous zinc chloride and methyl alcohol until the latter was saturated. The flask was then gently heated in a water-bath and the methyl chloride evolved was passed successively through three gas washers, the first two containing fuming sulphuric acid and the third distilled water. The methyl chloride was allowed to escape for an hour and a half, in order to free completely the apparatus from air, and was then collected in a gas-holder filled with air-free water. When a sufficient quantity had been collected it was shaken up with the water of the gas-holder and allowed to stand, in order to dissolve any trace of methyl alcohol present. Samples of gas were removed just prior to the experiments and measured in gas burettes over mercury. Contact with the mercury was limited to half an hour, since it had been found that contact for two weeks resulted in a reduction of the chlorine content by 30%.

Analysis of the Gases from the Catalyst Tube.

The chief problem in analysis of the effluent gases proved to be the quantitative hydrolysis of the methyl chloride. Complete hydrolysis of the methyl chloride and absorption of the hydrogen chloride produced by hydrolysis was found to take place on passing the gas over calcium hydroxide at 650°C., but unfortunately the decomposition products vitiated the subsequent methane determination. McKee (6) has found that some dimethyl ether is usually formed at 350°C. but that this can be prevented by the use of *moist* methyl chloride. This was tried and found satisfactory.

The gases from the catalyst tube were passed (i) through two gas washers containing 10% solutions of potassium iodide, to remove free chlorine and hydrochloric acid; (ii) through water at 95°C. to humidify them preparatory to hydrolysis; (iii) over chloride-free calcium hydroxide at 350°C., with the formation of methyl alcohol and calcium chloride; (iv) to a gas-holder containing water, in which they were allowed to remain a few hours for the absorption of the methyl alcohol; (v) through concentrated sulphuric acid, to dry them; (vi) over iodine pentoxide at 125°C., to oxidize carbon monoxide, the iodine being removed by passage over heated copper gauze, according to Boswell's modification (2) of the method of Levy (5); (vii) through potassium hydroxide solution and sulphuric acid for the removal of carbon dioxide and

water; and, finally, (viii) through a combustion tube containing copper oxide, followed by sulphuric acid and potassium hydroxide, for the determination of the methane. After the experiment was completed the potassium iodide solution was first titrated with sodium thiosulphate to determine the unused chlorine, then with sodium hydroxide, to determine the hydrogen chloride evolved. The chlorine absorbed by the lime was determined by solution in nitric acid and precipitation by means of silver nitrate. The method of analysis, therefore, provided for the determination of the volumes of chlorine and methane unused, the volume of hydrogen chloride formed and the weight of chlorine in the chlorinated products other than hydrogen chloride. The accuracy of the method was demonstrated by the passage of mixtures of known volumes of the gases.

The Chlorination of Methane on an Analytical Scale

The Generation of Methane.

Methane was generated by the action of water on aluminium carbide. The gas was purified by passing it through fuming sulphuric acid and water. It was analyzed by the usual volumetric methods and also gravimetrically by combustion over copper oxide, with the following results: methane 92%; hydrogen 8%.

Preparation of the Catalyst.

The catalyst was prepared by impregnating 18 gm. of pumice, which had been passed through a 12-mesh sieve with a solution of 10 gm. of cupric chloride ($CuCl_2 . 2H_2O$) in 15 cc. of water. After being dried at 110°C., it was placed in a hard glass tube 17 mm. in diameter, and was heated in an electric furnace at 450°C. for nine hours, while moist nitrogen was passed over it. Previous experiments had shown that by this treatment the cupric chloride is reduced about three quarters of the way to the cuprous condition, with the evolution of chlorine and oxygen.

Apparatus and Procedure.

The apparatus shown in Fig. 1 was used, with only minor changes, throughout the whole series of experiments.

The nitrogen used in diluting the methane and chlorine was passed successively through J, where it was heated to 750° C. for the removal of oxygen and the oxidation of any organic material; through K, to remove any carbon dioxide; through L, to moisten it (beginning with experiment 19); and then through the train of apparatus. The gas washers P and P_2 were filled with a 10% solution of potassium iodide, Q and Q_2 were half-filled with distilled water, R and R_2 were filled with chloride-free lime and V with water. The nitrogen was allowed to bubble out through T until required. The burette E was filled with dry methane and left under a pressure of a few centimetres of mercury when filled to the 100 cc. mark. Chlorine from A was

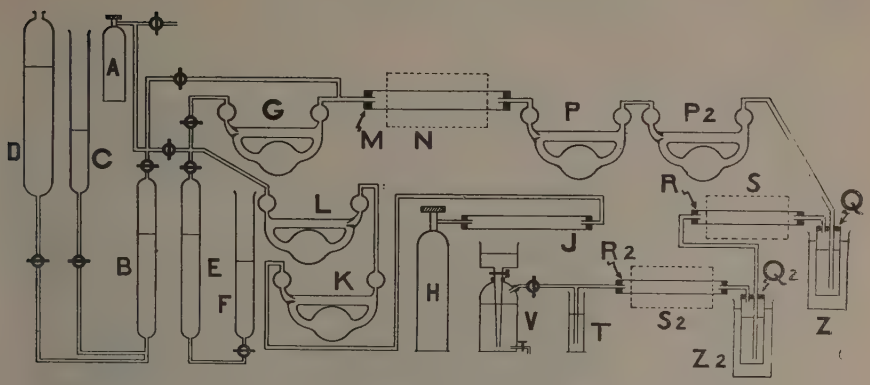

FIG. 1. APPARATUS FOR THE CHLORINATION OF METHANE ON AN ANALYTICAL SCALE.

A: Chlorine cylinder. B: Water-jacketed gas burette. D: 1000 cc. separatory funnel, placed about 6 ft. above B. C: Levelling tube. E: Mercury-filled, water-jacketed gas burette. F: Levelling tube at a higher level. G: Gas washer containing conc. H_2SO_4. H: Nitrogen cylinder. J:Silica tube containing Cu and CuO heated to 750° C. K: Gas washer containing 1:1 KOH solution. L: Gas washer containing water. M: Tube containing the catalyst. N: Electric furnace. P, P_2: Gas washers containing KI solution. Q, Q_2: Humidifiers. Z, Z_2: Water baths to maintain Q and Q_2 at 95° C. R, R_2: Tubes containing lime. S, S_2: Electric furnaces. T: Water manometer. V: 10-litre gasholder.

passed through B and bubbled up through the water in D until required. The temperature of M was raised to 450° C., of R and R_2 to 350° C., and of the water baths Z and Z_2 to 95° C. Then the chlorine was shut off and water was allowed to flow from D into B until it reached the 100 cc. mark, leaving this volume of chlorine under pressure. The pinch-cocks at the top of the burettes E and B were then opened to the atmosphere for an instant, leaving in them 100 cc. of methane and chlorine, respectively, at atmospheric pressure and room temperature, the remainder of the apparatus being filled with nitrogen. The passage of methane and chlorine was then commenced, the rates being adjusted by means of pinch-cocks in the tubing supplying mercury and water, respectively, to the burettes. The methane-chlorine-nitrogen mixture could be dried, if desired, by passage through G. The passage of chlorine through the water in D, prior to the experiment, made certain that none of the measured sample of chlorine would dissolve in the water of the gas burette. As soon as the passage of methane and chlorine was commenced, the exit gases were collected in the gas-holder, atmospheric pressure being maintained in the system as indicated by the manometer T. The passage of nitrogen was continued for one and one-half hours after all the methane and chlorine had been passed, in order to ensure the collection of all the products of the reaction. While the nitrogen was still passing, the few drops of water which usually collected in the cold end of the reaction tube were driven over into P by gentle heating. The contents of P and P_2 were analyzed and after standing for two hours, the unused methane in the gas in the gas-holder was determined, as already described.

TABLE I

CHLORINATION OF METHANE TO METHYL CHLORIDE ON AN ANALYTICAL SCALE

Variables	Preliminary		Temperature			Chlorine and Temperature				Nitrogen		
Experiment No.	17	18	19	20	21	22	23	24	26	27	28	29
Temperature, in degrees C.	450	450	450	420	475	450	420	450	450	450	450	450
Time, in minutes	15	15	15	15	15	15	15	15	15	15	15	20
Gases passed:												
CH_4, cc.	88.0	86.7	86.5	85.6	86.3	85.5	85.6	84.6	86.2	86.2	85.9	86.1
Cl_2, cc.	122.9	94.6	93.7	92.7	93.3	45.9	46.5	30.5	31.1	30.4	29.8	30.4
Ratio, N_2:CH_4										3	6	10
Condition of gases	Dry	Dry	Moist	Moist	Moist	Moist	Moist	Moist	Moist	Moist	Moist	Moist
Determined:												
Gases unused, CH_4, cc	61.8	26.6	23.6	34.8	29.0	38.3	53.3	43.4	43.1	76.0	58.2	59.7
Cl_2, cc.	0.0	21.2	21.8	0.0	2.8	0.1	trace	2.6		0.0	0.0	0.0
Cl_2 converted to HCl, cc.		61.5	20.0		28.3	5.3	1.3	3.8		7.4	2.2	7.4
Cl_2 in other products, cc.	18.3	53.2	54.8	45.0	52.7	35.1	26.8	21.3	20.7	16.0	20.0	12.3
H_2 in exit gases, cc.		24.7		30.4	27.2	16.0	14.1	22.7	14.6	3.1	0.0	0.0
Calculated: (in vapor phase)												
CH_4 used, cc.	26.2	60.1	62.9	50.8	57.3	47.2	32.3	41.2	43.1	10.2	27.7	26.4
CH_3Cl produced, cc.	15.8	13.8	16.2	11.6	9.3	24.2	11 0	39.8	41.4	0.0	15.4	24.6
CH_2Cl_2 produced, cc.	10.4	46.3	46.7	39.2	48.0	23.0	21.3	1.4	0.0	0.0	12.3	0.0
$CHCl_3$ produced, cc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.8	0.0	0.0
CCl_4 produced, cc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0	0.0
Ratio, CH_3Cl:CH_2Cl_2	1.5	0.29	0.34	0.29	0.19	1.05	0.53	29			1.25	
Percentage yield of CH_3Cl, based on CH_4 used	60	23	26	23	16	51	34	97	100	0	56	100

Effect of Moisture in the Gases.

In the preliminary experiments the reacting gases were thoroughly dried before entering the catalyst tube. The result obtained when the catalyst was freshly prepared (i.e., in the reduced condition) was the absorption of a large volume of chlorine by the catalyst (experiment 17, Table I). Before proceeding to experiment 18, the catalyst was thoroughly saturated with this gas, and any excess of chlorine was swept out with nitrogen. In this experiment considerably more chlorine appeared in the products than was passed, showing that it had been given off by the catalyst. In order to avoid these difficulties the gases were saturated with moisture before entering the catalytic tube, with the result that the catalyst was maintained in the partially reduced and *constant* condition very favorable to the reaction. This constancy in condition of the catalyst is obviously highly desirable in quantitative experiments.

Effect of Reaction Temperature.

The most favorable temperature was believed to be 450° C., but experiments were also carried out at 420° and 475°C. By comparing the results of experiments 19-21 (Table I) it will be seen that the yield of methyl chloride was greatest at 450°C. A similar, but more marked, result is seen in experiments 22 and 23 where lower ratios of chlorine to methane were used.

The Methane-Chlorine Ratio.

The volumes of methane and chlorine in the reacting mixtures were approximately equal in experiments 17-21, but that of chlorine was reduced by 50% in the two succeeding experiments by mixing with it in the burette an equal volume of nitrogen. In the remaining experiments recorded in Table I, the methane-chlorine ratio was approximately three to one. It will be observed that, other things being equal, the yields of methyl chloride improved as the proportion of chlorine was reduced. In experiment 17, where a large volume of chlorine was passed, the good yield is doubtless to be accounted for by the fact that much chlorine was absorbed by the catalyst, none being found in the exit gases.

Nitrogen-Methane Ratio.

In the earlier experiments the volume of nitrogen was not measured, as its importance was at that time not fully realized. Later, however, a series of experiments was carried out to determine the effect of dilution with a large volume of nitrogen, upon the yield of methyl chloride. It will be noticed from Table I that the yield increased from zero to 100% as the nitrogen-methane ratio increased from 3:1 to 10:1. In experiment 29 (as also in No. 26) the yield is recorded as 100% because the evidence of complete conversion to methyl chloride was conclusive; the excess of methane used over methyl chloride produced is accounted for in both cases by the solubility of methane in water. For the same reason, it is believed that all the results recorded as "methane used" are slightly too high.

Effect of Hydrogen in the Methane.

As already stated, the methane used in this series of experiments contained 8% of hydrogen. In determining the yield, based upon the methane used, allowance was made for this dilution of the methane. So also, in calculating the methane-chlorine ratio, the volume of chlorine theoretically required to unite with the hydrogen of the methane to form hydrogen chloride was deducted, since this reaction was known to take place rapidly under the conditions of the experiments.

In spite of this reaction tendency, a considerable percentage of hydrogen was found in the residual gases. Further, the results given in Table I show that in most of the experiments much less than half the volume of the chlorine passed was contained in the hydrogen chloride evolved, and much more than half in the other chlorinated products.

It may be that the hydrogen came from the hydrogen chloride unaccounted for, the equivalent chlorine producing the observed excess of chlorinated products. On the other hand, it may have been derived from the action of lime and steam on the methyl chloride, but experiments indicated that this reaction did not take place. No explanation of these anomalies is offered.

The Chlorination of Methane on a Larger Scale

Apparatus and Procedure.

Having determined on an analytical scale the conditions under which methane can be chlorinated to produce only methyl chloride, with complete utilization of the chlorine, the next step was to carry out the chlorination on a scale sufficiently large to permit the collection and measurement of the products. Accordingly, an apparatus designed to produce 25 gm. of methyl chloride per experiment was constructed and set up.

As before, the methane was generated by means of aluminium carbide. In this case, however, ice was used instead of water, in order to delay the reaction. In a small autoclave were placed 288 gm. of powdered aluminium carbide and 432 gm. of crushed ice, and the cover of the autoclave was immediately bolted on. When the pressure rose to about 150 lb. per sq. in. the valve was opened for a moment to blow off most of the small quantity of residual air. Upon closing the valve the pressure quickly rose to 6000 lb. per sq. in., and then fell off to 4500 lb. per sq. in. on cooling. This provided 60 litres of methane under pressure. Analysis—made after experiment 43 had been completed—showed the gas to contain 18% of hydrogen. The effect of this hydrogen will be discussed later.

The catalyst was prepared as before, 173 gm. of $CuCl_2.2H_2O$ being used. The gases were moistened in order to maintain the catalyst in the proper condition. The reaction chamber in which the catalyst was placed consisted of a silica tube 60 cm. long and 5 cm. inside diameter, wound with resistance wire, and water-cooled at both ends. The effective volume was 17 times as great as in the previous experiments, making possible the passage of methane at the rate of 6000 cc. per hour.

The apparatus used in this series of experiments is shown in Fig. 2. The gas holders A, A_2 and A_3 were filled with methane, nitrogen was turned on at the desired rate, and chlorine was supplied at constant pressure by allowing a little to escape continuously through E. The gases from the reaction tube were passed through soda-lime and (in some of the experiments) calcium chloride in the tower S. The chlorinated products were condensed by passing them into large test tubes surrounded by liquid air in Dewar flasks.

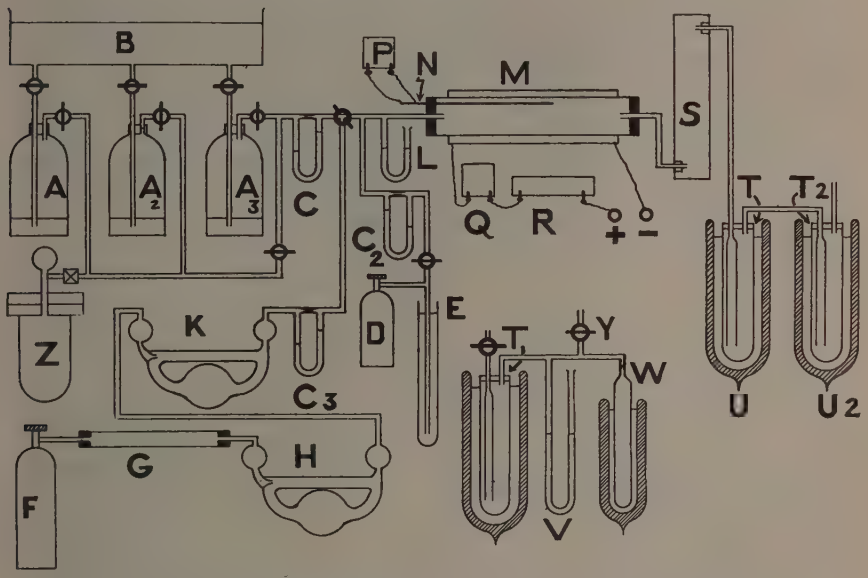

FIG. 2. APPARATUS FOR THE LARGER-SCALE CHLORINATION OF METHANE.

A, A_2, A_3: 15-litre gas holders, calibrated on the outside. B: Large water reservoir. C, C_2, C_3: Capillary flow-meters. D: Chlorine cylinder. E: Tube about 2 ft. long containing water, with delivery tube running nearly to the bottom. F:Nitrogen cylinder. G: Silica tube containing Cu and CuO at 750° C. H: Gas washer containing 1:1 KOH. K: Gas washer containing water. L: Water manometer. M: Reaction tube, wound with resistance wire. N: Thermocouple. P: Millivoltmeter. Q: Ammeter. R: Variable resistance. S: Tower containing soda-lime. T, T_2: Large test tubes with wide delivery tube leading to within about an inch of the bottom. U, U_2: Dewar flasks. V: Mercury manometer, capable of registering pressures from 15 mm. to 2 atmospheres. W: Carius tube drawn to capillary at the top. Z: Autoclave.

The experiments required about six hours each. When the methane and chlorine had all been passed, the system was swept out with nitrogen for about 15 minutes. The test tubes containing the liquefied products were then disconnected from the soda-lime jar and, after closing the exit tube of T_2 and opening the entrance tube of T, the liquid air was removed from around the second tube. The small amount of condensate was thereby distilled back into the first tube which was then connected to the mercury manometer and

to a calibrated Carius tube drawn down to a capillary, as shown in Fig. 2. The liquid air surrounding the tube was removed and a mixture of carbon dioxide snow and ether (−79°C.) was substituted for it. By this procedure any condensed methane was boiled off (b.p. —164°C.). The quantity was always small. A little methyl chloride was lost in this way, so that the weights recorded (Table II) are slightly below the actual yields. The system was then evacuated through Y and the pinch-cock at Y was closed. The methyl chloride in the test tube was then distilled over and condensed in the Carius tube which was surrounded by liquid air. When distillation was complete, the Carius tube was disconnected from the remainder of the apparatus, and, while still immersed in liquid air, the tip was sealed by a blow-torch. It was subsequently warmed to 0°C., the volume of methyl chloride was measured at this temperature, and its weight calculated. Any residue remaining in the test tube was fractionally distilled for the separation and determination of methylene chloride, chloroform, and carbon tetrachloride.

TABLE II

CHLORINATION OF METHANE TO METHYL CHLORIDE ON A LARGER SCALE

Experiment No.	35	37	38	40	43	46	47	48	49	50
Special purpose of Experiment		Better collection of products	Test of collecting system			Use of CH_4 freed from H_2	To confirm experiment No. 46	Effect of increased ratio of CH_4:Cl_2	To confirm experiment No. 48	To det. effect of faster passage of CH_4 and Cl_2
Time, hr.	6.17	5.25	6.33	7.00	5.33	5.68	5.63	6.00	5.67	2.47
Gases passed:										
CH_4, cc.	36150	30400	33275	44500	32350	32580	32000	32680	34400	35400
H_2 in CH_4, %	18	18	18	18	18	1	0	1.4	0	0
Net CH_4, cc.	29643	24928	27285	36490	26527	32255	32000	32222	34400	35400
Total Cl_2, cc.	12350	8950	10700	13300	9800	10457	10365	4740	4480	4065
Net Cl_2 (total less vol. required to unite with H_2 of CH_4 as HCl), cc.	5843	3488	4710	5290	3977	10132	10365	4280	4480	4065
Effective ratio, CH_4:Cl_2	5.1	7.1	5.8	6.9	6.7	3.2	3.1	7.5	7.7	8.7
Ratio, N_2:CH_4	10	10	10	10	10	10	10	10	10	5
Produced:										
CH_3Cl, gm.	8.6	6.8	6.9	11.4	7.6	10.9	11.4	7.6	7.4	6.9
CH_2Cl_2, gm.	Trace	Trace	0.0	2.5	Lost	5.0	5.5	1.0	1.2	0.5
$CHCl_3$, gm.	0.0	0.0	0.0	0.0	2.0	0.2	0.5	0.0	0.0	0.0
CCl_4, gm.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chlorinated products apparently lost, %						25	23	9	12	18.5
Percentage yield of CH_3Cl:										
Indicated	31	34	29	38	36					
Effective based on net Cl_2 passed	65	87	65	96	84	48	48	79	74	75

Efficiency of Collection Apparatus.

In experiment 35, the indicated yield of methyl chloride was found to be only 31% (Table II). For this reason the efficiency of the collecting apparatus was carefully tested (Experiments 37 and 38). In the former experiment the exit gases were bubbled through liquid air; in the latter the gases leaving the test tubes were passed over chloride-free calcium hydroxide at 600°C. for the hydrolysis of any escaping methyl chloride, the lime being subsequently treated, as in the experiments on an analytical scale, for the determination of chlorides. The calibration of the Carius tube was also checked by weighing the methyl chloride collected. This was done by weighing the sealed Carius tube, cooling in liquid air, breaking off the tip carefully, allowing the methyl chloride to evaporate on warming the Carius tube to room temperature, and weighing tube and tip again. These experiments showed that no appreciable amount of methyl chloride was being lost in the regular procedure.

Another experiment (No. 39) was carried out to determine whether free chlorine was escaping from the reaction tube, since that, although unlikely, would account for the low yield of methyl chloride obtained. The result was negative.

Effect of Hydrogen upon the Reaction.

Experiments 35-43 (Table II) were performed in the belief that, as in the first series of experiments, the methane contained 8% of hydrogen. The indicated yields of methyl chloride, however, were so out of harmony with previous results as to point to a relatively greater amount of hydrogen, which would combine with part of the available chlorine. This conjecture was confirmed by analysis, which showed a hydrogen content of 18%. This amount of hydrogen in the methane would, with a methane-chlorine ratio of three to one, combine with 54% of the chlorine present. In subsequent experiments the hydrogen was therefore removed by passage over heated copper oxide at 275°C., since Burrell and Oberfell (3) have shown that this results in the selective oxidation of hydrogen in the presence of methane. The gas was passed through a 1:1 potassium hydroxide solution before being collected.

The yields of experiments 35-43 were then recalculated on the basis of the net chlorine present. The results showed that some of the yields were of the high order anticipated, as may be seen by reference to Table II.

Experiments 46 and 47 were then carried out with the purified methane. The indicated yield of methyl chloride increased considerably, but at the same time more than a third of the chlorine appeared as methylene chloride and chloroform. Further, there was an indicated loss of about 25% of the products of chlorination.

Methane-Chlorine Ratio.

It was then realized that, in these two experiments, the ratio of methane to chlorine was too low, and the effective ratios of these two gases in the earlier experiments were accordingly calculated. The results, shown in

Table II, revealed the fact that in experiments 46 and 47 the effective ratios of methane to chlorine were less than half those previously used. The reduced mass action of the smaller quantity of hydrogen chloride present had evidently permitted the formation of the higher chlorinated products.

In experiments 48 and 49 the methane-chlorine ratios were therefore raised to 7.5 and 7.7, resulting in the very satisfactory yields of 79% and 74% respectively, with only a small proportion of methylene chloride in either case. Further, the percentage of chlorinated products apparently lost decreased to 9 and 12. As the boiling point of methylene chloride (42°C.) is considerably higher than that of methyl chloride (−23.7°C.) it may be assumed that the losses consisted chiefly of the latter, and that the total yields in these two experiments were actually in the neighborhood of 88% and 85%, respectively.

Rate of Passage of Gases.

In this series of experiments the rate of passage of the gases had been kept down to a point found by the experiments on an analytical scale to be within the limit necessary for satisfactory chlorination. In experiment 50, however, the rates of passage of methane and chlorine were doubled to determine what effect this might have upon the efficiency of the catalyst. The rate of passage of the nitrogen was not changed, and the nitrogen-methane ratio was therefore reduced by half. The yield of methyl chloride condensed was 75%, which was practically the same as at the slower rate of passage, in spite of the increased loss of chlorinated products (18.5%). If this loss represents methyl chloride the gross yield was over 90%.

Optimum Conditions for Chlorination to Methyl Chloride

The conditions recommended for the chlorination of methane exclusively to methyl chloride are as follows:

1. Nitrogen-methane ratio, ten to one. This can be reduced somewhat, under favorable conditions, without appreciably lowering the yield of methyl chloride.

2. Methane-chlorine ratio, seven or eight to one. If methane containing 8 % of hydrogen be used this can be reduced to three to one.

3. Condition of gases, moist.

4. Temperature of reaction, 450°C.

5. Catalyst, partly reduced cupric chloride on pumice.

Under these conditions it has been found possible to chlorinate methane to methyl chloride with a yield based on chlorine passed, of 75-80% or higher. A small quantity of methylene chloride is also formed.

Chlorination of Methane to Carbon Tetrachloride

An attempt was now made so to control the chlorination of methane as to produce carbon tetrachloride exclusively. The apparatus, shown in Fig. 2, was exactly the same as that formerly used. Fresh catalyst was prepared in the same manner as before. Carbon dioxide snow in ether was used as the condensing medium, in place of liquid air. The results of this series of experiments are shown in Table III.

TABLE III

CHLORINATION OF METHANE TO CARBON TETRACHLORIDE

Experiment No.	51	52	53
Time, hr.	6.0	7.0	6.5
Gases passed:			
CH_4, cc.	3,270	3,720	6,280
Cl_2, cc.	12,000	14,000	26,000
N_2, cc.	none	none	47,000
Produced:			
CCl_4, gm.	18.6	21.8	34.5
$CHCl_3$, gm.	none	none	3.0
Higher-boiling liquid, gm.	trace	1.5	1.0
Yield of CCl_4, %	90·3	90.8	80.2

In experiment 51 methane and chlorine were passed in approximately theoretical proportions (1:4) without nitrogen. Nitrogen was, however, passed through the apparatus while the reaction tube was being heated up, and again at the end of the experiment, to sweep out the products. The methane was freed from hydrogen before use. The carbon tetrachloride obtained represented a yield of 90%. Its purity was tested by fractional distillation. There was no indication of chloroform or other low-boiling compounds. A slight residue of liquid boiling over 100°C. was obtained.

Experiment 52 was a duplicate of the above. The yield of carbon tetrachloride (90.8%) was slightly higher than before, as was also that of the higher-boiling liquid. (b.p. about 120°C.)

A third chlorination experiment (No. 53) was then performed to determine the effect of doubling the rate of passage of the gases. As soon as these gases entered the reaction tube an explosion took place, a flame shooting back in the apparatus and carbon being deposited. The passage of the gases was continued, but when the explosions were found to occur at frequent intervals nitrogen was introduced. This decreased the frequency of the explosions, but they did not cease until the volume of nitrogen was seven times that of the methane. The data recorded for experiment 53 in Table III apply to the experiment as continued from this point.

The three grams reported as chloroform (b.p. 61.2°C.) consisted of all the distillate between 65° and 74°C., hence it no doubt contained considerable carbon tetrachloride (b.p. 76.7°C.). The yield of the latter, shown as 80.2%, was therefore probably somewhat higher. This yield, though not as good as at the slower rate of passage, was nevertheless considered very satisfactory.

The Chlorination of Ethane to Ethyl Chloride

The chlorination of ethane is also of considerable interest, since this gas is an important constituent of some natural gases. For example, the natural gas used by the city of Pittsburgh, Pa., contains 10% of ethane, and that of one well in the Turner Valley oil and gas field, in southern Alberta, as much as 66%.

Ethane may be regarded as a potential source of ethyl alcohol, the first step in the production of the alcohol being chlorination to ethyl chloride, and the second, hydrolysis. Conditions suitable for the chlorination of methane to methyl chloride having been found, a preliminary study of the chlorination of ethane was made.

It was found that the procedure used in the chlorination of methane on an analytical scale would have to be considerably modified for the chlorination of ethane in order to make possible a calculation of the yield. Only a few experiments were performed, and no accurate data regarding these are available, but the yield of ethyl chloride, calculated from the ethane and chlorine used, was at least 75%. It is the intention of the authors to investigate further the chlorination of ethane on a scale sufficiently large to permit the accurate measurement of the ethyl chloride formed.

Commercial Considerations

The results of the experiments outlined above are presented as essential laboratory data upon which larger-scale experiments may be based. Much further work will be required to determine whether the methods used will be applicable on a commercial scale. Certain economic considerations may nevertheless be of interest.

The necessity for great dilution of chlorine with nitrogen and methane in the production of methyl chloride might, at first sight, be considered a grave objection to any commercial process based upon these laboratory methods. However, this is not necessarily the case for, after the removal of the hydrogen chloride and methyl chloride produced, the excess methane and diluent nitrogen can be recirculated through the apparatus, after the necessary addition of chlorine and methane. Thus, neglecting mechanical losses, it would be necessary to supply nitrogen to the system only at the outset.

If the hydrogen chloride formed were removed by lime, calcium chloride, of low commercial value, would be produced. However, if the hydrogen chloride were passed through a suitable water washer, it might be possible to recover most of it as a solution, without any appreciable loss of methyl chloride.

A possible change in the process on a commercial scale would be the substitution of methane for the diluent nitrogen.

In the chlorination of methane to carbon tetrachloride the tendency of the reacting gases to explode as their rate of passage is increased can be overcome by dilution with nitrogen, although this results in the production of some chloroform. The explosions are caused by the heat of reaction; it might

therefore be possible so to regulate the speed of the undiluted reacting gases that the temperature of the catalyst would just be maintained at 450°C., without any external heating. The apparatus could also be so modified, that the gases would not mix until they entered the reaction chamber, thus effectively preventing serious explosions, whatever the rate of passage.

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POTASSIUM NITRATE IN CANADIAN CHEESE[1]

By F. C. Harrison[2]

Abstract

The addition of saltpetre (potassium nitrate) to colored cheese is shown to produce discoloration which is unevenly distributed and which becomes very marked with age. Fresh nitrated cheese gives a positive test for nitrates; on aging, the nitrite is decomposed. Nitrate-reducing organisms have been isolated from all discolored cheese. These, when inoculated into sterilized milk with annato and potassium nitrate, produce the typical flesh-colored or vinaceous-brown discoloration. Commercial saltpetre may contain nitrate-reducing organisms, as may also the water used in diluting rennet or cheese colors. *Pseudomonas fluorescens*, one water organism capable of producing the discoloration, was isolated. It is recommended that the use of saltpetre in cheese be immediately discontinued.

Introduction

In recent years certain cheese factories have been adding saltpetre (potassium nitrate) to the cheese curds at the time it goes to press; the amount used varies, but the usual rate is six ounces of saltpetre to one thousand pounds of milk. Cheesemakers consider that this chemical temporarily restrains or controls certain injurious fermentations, but it will be shown in this paper that when the ultimate result on the cheese is considered, the consequences are decidedly harmful.

There are few references to the use of saltpetre in Cheddar cheese, but some work has been done on the use of this chemical in the manufacture of the well-known Swiss, Gruyère or Emmenthaler cheese, with its characteristic holes and sweet flavor, and also on Edam (Dutch) cheese.

Orla-Jensen (4) believes that saltpetre is an excellent preventive of the harmful effects of such bacteria as the *aerogenes* and *colon* organisms in amounts of 20 to 30 grams of potassium nitrate to 100 litres of milk. He explains the beneficial action thus: "These organisms are able not only to effect respiration by means of atmospheric oxygen, but they will transfer loosely bound oxygen from oxidising agents like saltpetre, to sugar, and thus consume the sugar completely, so that no hydrogen is liberated or lactic acid formed".

Wolff and Berberich (6) made Swiss cheese from milk to which saltpetre had been added at rates varying from 20-60 grams per 100 litres. They found that the formation of holes in the cheese was decreased. Cracks developed in six weeks which they attributed to micro-organisms in the saltpetre used.

Haglund (3) reported experiments which showed that the addition of saltpetre to milk used in the production of Swiss cheese decreased the content of volatile fatty acids, especially propionic acid, the formation of which from lactic acid by the bacteria concerned is associated with the formation of holes

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in the cheese. Such experimental cheese were "blind", that is, they had no holes or eyes, were discolored, and not infrequently had a more or less decided taste of saltpetre.

Boekhout, van Dam and van Beynum (2) found that a characteristic brownish-red to greenish-yellow rind, from a quarter to several centimetres thick, was formed on cheese to which saltpetre had been added, and that such formation ran parallel to the content of nitrate produced from the added saltpetre. Bacteria which reduced nitrates to nitrites were isolated. These writers state that nitrogen and carbon dioxide are formed by the action of nitrates on casein, and that the coloring matter in cheese showed no evident influence.

Boekhout and de Vries (1) found that an organism which produced the huffing or gas holes in Edam cheese was prevented from growing by adding saltpetre to whey gelatin and that gas production was very considerably lessened. They suggested the use of saltpetre for controlling this fermentation.

Experimental

During the season of 1928 considerable trouble was experienced with discoloration of colored Canadian cheese, some of which was rendered unsaleable. Samples were examined, and showed both marked discoloration and objectionable flavor. The color, instead of being a light orange-yellow, was a peculiar reddish flesh color towards the centre, and nearer the ends was dirtier and muddier, comparable to the color described by Ridgeway as "vinaceous-cinnamon". The samples were examined bacteriologically and a number of organisms were isolated. In the outer portions of the cheese there were numerous moulds, especially *Monilia sitophylla*, which gives a characteristic pink color when growing on the usual media. *Aerobacter aerogenes* and *E. coli* were present, and a number of cocci. In some of the earlier experiments, cultures of the *Monilia* were made on uncolored cheese, resulting in the development of a certain amount of color. This organism, when grown in sterilized milk to which annato cheese color had been added, seemed to have the ability of taking up the color, and producing, by contrast with the remainder of the colored milk, a more intense red where the mycelium was growing. In other words, the mould stained more deeply than the colored milk.

Results secured later suggested that saltpetre had been used in the cheese, hence the next series of experiments was carried out in milk to which saltpetre had been added. Certified milk and skim milk were tubed and flasked, then sterilized. An amount of annato cheese color slightly greater than that used in cheesemaking, and 0.05 to 0.1% of potassium nitrate were added to each flask or tube. These were then inoculated with the organism obtained from the original discolored cheese, and also with an emulsion of this cheese. Uninoculated controls were kept of each series.

Indications of color change were noticeable in a few days; in some tubes the characteristic flesh color was present on the surface and became more marked with age. Table I shows the results obtained. All tubes were kept at room temperature.

TABLE I.

MILK IN FLASKS AND TUBES WITH ADDED ANNATO AND POTASSIUM NITRATE.

1. Control cultures.	No discoloration. Orange-yellow.
2. Emulsion of cheese.	Pink, flesh color in three days, increasing in seven days, moulds appearing on surface. Color below still pink.
3. A coccus isolated from original cheese.	Pink flesh color in three days—increasing with age.
4. *Bacillus subtilis.*	No change of color.
5. Lactic acid bacillus.	Coagulation, lighter in color but no discoloration.

The coccus was re-isolated from No. 3, and a second passage through nitrated colored milk gave similar results. These few experiments demonstrated that the color was due to the action of micro-organisms in nitrated milk.

Other samples of discolored cheese were obtained and put through the same tests, and no difficulty was experienced in obtaining discoloration in nitrated colored milk from emulsions of such cheese. In some instances the action was delayed, hence a special technique was devised which gave rapid and reliable results.

It was evident from these preliminary experiments that the nitrate played an important part in the discoloration, and it was thought that in all probability the reduction of nitrate to nitrite was instrumental in producing the color change. If this were true, then the nitrite test should give indications that saltpetre had been added to the milk or cheese, and the use of nitrate broth would be helpful in quickly isolating the causal organisms.

The first tests of discolored cheese, however, were all negative, even when large samples were triturated in sterile water and then filtered. The filtrate did not give any reaction to the sulphanilic acid and dimethyl a. naphthalene test. All the nitrite had evidently been converted into ammonia or free nitrogen.

Samples of this season's cheese (1929), however, gave an immediate positive reaction, and when the presence of saltpetre is suspected this test is recommended as a routine procedure. It is only necessary to grind up part of a cheese plug in sterile water, and test at once, or to filter and test the filtrate; the latter, however, is not absolutely necessary.

From a series of tests and observations, the best results for isolating the organisms responsible for the color change were obtained by:

1. Culturing a few loopfuls of the cheese emulsion in potassium nitrate broth.
2. Plating out a dilution of the cheese in ammonium phosphate agar with brom cresol purple as indicator.

In method 1, after 24 to 48 hours' growth, a drop of the indicator is placed on a white porcelain plate and a loopful of the culture is added; if the reaction is pink, the culture is plated out in ordinary agar and isolated. Both methods have been used together and give quick and reliable results.

As soon as colonies appear on the plates they are isolated and subcultured in nitrate broth. The broth is tested daily for nitrite, and, if positive, nitrated annato milk is inoculated, kept at room temperature, and color indications observed.

By these methods a number of samples of cheese have been examined, nitrate-reducing organisms isolated, and tests made in nitrated annato milk. The organisms listed in Table II have been isolated, identified, and the result of their growth in nitrated annato milk ascertained.

TABLE II.

ORGANISMS IN SKIM MILK, WITH ADDED ANNATO AND POTASSIUM NITRATE

Organism	Effect on nitrated annato milk.
Sta. pyogenes aureus	Flesh-colored, reduction.
Sta. pyogenes albus	Flesh-colored, reduction.
Micrococcus varians	Some strains gave a positive and some a negative discoloration.
Lactobacillus acidophilus	No change in color.
Escherichia coli	Flesh color, reduction; with age dirtier and more bleached.
Escherichia communior	Flesh color, becoming dirtier with age.
Escherichia neapolitana	Very marked flesh color, later becoming lighter.
Aerobacter aerogenes	Flesh-colored, then dirtier.
Aerobacter cloacae	Flesh-colored, and rather red.
Proteus sp.	Flesh-colored.
Pseudomonas fluorescens	Flesh-colored, reduction.
Monilia sitophylla	Positive, reduction.

All the above organisms reduce nitrates to nitrites in nitrate broth, but not all produce the peculiar flesh color. The lactic acid organisms like *Strept. cremoris*, *Strept. lactis*, and *Lacto b. acidophilus* do not produce the color change.

As Wolff and Berberich (6) had found nitrate-reducing organisms in potassium nitrate, it was thought advisable to find out if such organisms were present in commercial samples of saltpetre. Several samples from different sources were obtained, and 10% solutions made up in sterilized water. After standing for some days, a few drops from each solution were transferred to nitrate broth, allowed to stand at room temperature for two to four days, and then tested for nitrite. All samples gave positive tests, and nitrite-producing bacteria were isolated from the broth.

Certain moulds seemed to grow freely in the 10% saltpetre solutions.

It is well-known that the pickling solutions containing saltpetre, used in curing bacon and ham, very quickly swarm with bacteria, but these solutions do not contain such a large percentage of saltpetre. The reddening of pickled beef may be compared to the reddening of the cheese and may also have been caused by the reduction of the nitrate.

The chemistry of annato is not well known, but it is in all probability an unsaturated compound, which changes its color in the presence of acids and nitrogen compounds. Solutions of sodium nitrite will not effect any change, but in the presence of acid the color becomes redder. If milk to which annato has been added, to give an orange-yellow, be treated with a little sodium nitrite in solution, no change results; but, when a little acid is added, there is a very noticeable change of color in about 15 minutes. Hence, in all probability, other conditions besides the presence of nitrate-reducing bacteria are necessary to produce the color change in cheese, to which saltpetre is added.

Mention has been made of the fact that the saltpetre is added to the curds, and not to the milk. This would bring about an uneven distribution of the chemical in the cheese, and would account for the mottling, and the patchy production of the flesh color.

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STUDIES IN ISO-UREAS AND ISO-UREIDES

I. SOME NEW ISO-UREAS; SALTS AND ACYL DERIVATIVES[1]

By Steward Basterfield[2] and Edward C. Powell[3]

Abstract

In this investigation a series of new iso-urea ethers has been prepared by the general reaction of addition of alcohols to cyanamide in the presence of dry hydrogen chloride. Where the hydrochlorides of the iso-ureas were difficult to isolate and purify, conversion to the salicylates gave excellent results. The new iso-ureas were further characterized by condensing them with methyl malonate to form iso-urea salts of 2-alkoxy-barbituric acids, from which the acids themselves could be easily liberated by the addition of dilute mineral acid. A few open-chain acyl derivatives were also prepared.

Introduction

The chemistry of methyl- and ethyl-iso-ureas and their acyl derivatives has been studied mainly by Stieglitz and his collaborators (2, 3, 4), by Wheeler and Johnson (8), and by E. A. Werner (6). More recently Basterfield and Whelen (1) made a further contribution to this field of study in their work on n-propyl and n-butyl iso-ureas and their acyl derivatives.

In the present investigation a further study of n-propyl and n-butyl iso-ureas was made and in addition the possibility of preparing iso-propyl, iso-butyl, iso-amyl, tertiary amyl, benzyl, phenyl-ethyl, allyl and chloro-ethyl iso-ureas was investigated.

Experimental

The method of preparation of iso-urea hydrochlorides used was that of McKee (4) with modifications. This consists essentially in the addition of absolute alcohols to cyanamide in the presence of dry hydrogen chloride at 0°C. When the reaction for cyanamide has disappeared, the alcohols are distilled off under reduced pressure, leaving the hydrochlorides of the different iso-ureas as oils which may solidify wholly or partially when cooled in ether to temperatures from $-10°$ to $-20°$C.

The hydrochlorides of n-propyl, iso-propyl, n-butyl, iso-butyl, iso-amyl, and tertiary amyl iso-ureas were obtained as oils which only partially solidified in ether at $-10°$C. Some of these oils on standing in a vacuum over long periods of time formed sticky semi-crystalline sugary masses, which were very hygroscopic and difficult to handle, due perhaps to the fact that the removal of the last traces of alcohol was very difficult. The others remained as very viscous oils which were brown or yellow in color.

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Contribution from the laboratories of the University of Saskatchewan, Saskatoon, Canada. This paper is the first of a series and is based on a thesis presented by the junior author in partial fulfilment of the requirements for the degree of M.Sc. at the University of Saskatchewan.
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The iso-urea hydrochlorides were converted into their respective free bases by suspending them in moist ether and treating with several moles of powdered caustic potash. The free bases which are also oils, unstable to vacuum distillation, were then neutralized in ether solution by a more complex acid such as salicylic acid, and salts were obtained which could be purified and studied. Further characterization of the iso-ureas was made by the formation of either open-chain acyl derivatives or cyclic acyl derivatives of the type of the methyl malonate condensation products as studied by Stieglitz and Basterfield (5). The latter found that ethyl iso-urea condensed with methyl malonate to form in good yield the iso-urea salt of 2-ethoxy-barbituric acid.

Ethylene chlorhydrin did not appear to add to cyanamide in the presence of dry hydrogen chloride, there being no appreciable decrease in the reaction for cyanamide after 38 days. This was confirmed when no free base was obtained after distilling off the excess of ethylene chlorhydrin and treating the light brown oil which was left, with caustic potash in moist ether.

Benzyl and phenyl-ethyl iso-urea hydrochlorides were undoubtedly formed, the reaction for cyanamide disappearing in about seven and six days respectively. They were apparently decomposed when the excess of benzyl and phenyl-ethyl alcohols was distilled off under reduced pressure. This was presumably due to the fact that the alcohols have very high boiling-points and that the temperatures necessary for their distillation were above the decomposition points of their respective iso-urea hydrochlorides. The following analysis of a crystalline substance produced in the distillation indicated that probably the hydrochloride of dicyanamide (melting point about 125° C.) was formed during the above decomposition:

Analysis:	N calculated for $(CN)_2.NH.HCl$	40.60%
	Found	41.18, 41.28%

To avoid this decomposition, ethylene chlorhydrin was used as a medium for reactions between cyanamide and benzyl and phenyl-ethyl alcohols. Equivalent weights of cyanamide and alcohol were dissolved in twelve times the molecular equivalent of chlorhydrin. Benzyl and phenyl-ethyl iso-urea hydrochlorides were obtained, the former as an oil and the latter as a mush of oil and crystals. The free bases were obtained from these as oils in small yield. They formed salicylates, but no acyl derivatives nor barbituric acid ethers were obtained. A further study of these iso-ureas will be made.

It is doubtful if allyl iso-urea hydrochloride was formed, although the reaction for cyanamide disappeared in about twenty days. No free base, however, could be obtained from the black, viscous oil left from distillation of the alcohol by treating it with caustic potash in moist ether.

Tertiary-amyl iso-urea hydrochloride was formed in a very slow reaction as a light brown oil, but appeared unstable, as it deposited white crystals of what was perhaps polymerized cyanamide, while standing in a vacuum. The free base was obtained in small yield. It would not form a salicylate, but condensed with methyl malonate. The condensation product, assuming that a

salt of 2-tertiary-amoxy-barbituric acid and tertiary-amyl iso-urea was formed, yielded, on analysis, a percentage of nitrogen which was slightly high. The substance was readily soluble in hot alcohol from which it separated, on cooling, in fine white crystals. Melting point, 184° C.

Analysis: N calculated for $C_{15}H_{28}N_4O_4$ 17.06%
Found 17.99, 17.71%

Some study was made of the rates of addition of different alcohols to cyanamide. Four grams of cyanamide was dissolved in twelve times its molecular equivalent of alcohol, and approximately 3.6 gm. of dry hydrogen chloride was added. In most cases part of the cyanamide was precipitated as the di-hydrochloride, but as the reactions proceeded it gradually disappeared. The mixtures were allowed to stand in corked flasks at room temperature (23°–25° C.) until the test for cyanamide was negative. Results of this experiment are shown in Table I.

TABLE I

ADDITION OF VARIOUS ALCOHOLS TO CYANAMIDE

Alcohol	Time of disappearance of precipitated di-hydrochloride of cyanamide*	Time of disappearance of reaction for cyanamide	Molar ratio of alcohol and cyanamide
N-propyl	4 hours 3 hours	5 days 2 days	12:1
Iso-propyl	3 hours	2 days	12:1
N-butyl	12 hours 7 hours 4 hours	6 days 5 days 1 day	12:1 12:1 12:1
Iso-butyl	12 hours	6 days	12:1
Iso-amyl	74 hours 30 hours	7 days 2 days	12:1 12:1
Tertiary-Amyl	96 hours	21 days	12:1
Benzyl	114 hours	7 days	12:1
Phenyl-ethyl	24 hours	6 days	12:1
Allyl**	24 hours	19 days	12:1
Ethylene Chlorhydrin (12 moles) solvent:			
Phenol**	—	35 days	2:1
Benzyl	—	8 days	1:1
Phenyl-ethyl	—	7 days	1:1

*Time of disappearance of the reaction for cyanamide varies with the amount of di-hydrochloride of cyanamide precipitated. The latter depends on the amount of hydrogen chloride added. A small excess of hydrogen chloride retards the reaction by increasing this precipitation. It is therefore advisable to avoid adding more than the required amount of hydrogen chloride.

**No iso-urea was obtained.

Methyl Iso-urea; Salts and Acyl Derivatives

1. *Salicylate of Methyl iso-urea*, NH: $C(OCH_3).NH_2.HOOC.C_6H_4.OH$.

An ethereal solution of three grams of methyl iso-urea was treated with its molecular equivalent of salicylic acid dissolved in ether. The salicylate was deposited as a white amorphous substance only after the mixture was stirred vigorously for some time with a glass rod. It was very soluble in alcohol, but insoluble in benzene, ether and chloroform. Melting point, 128° C. Yield, quantitative.

Analysis:	N calculated for $C_9H_{12}N_2O_4$	13.20%
	Found	13.23, 13.16%

2. *Phthalyl-di-(methyl-iso-urea)*, $C_6H_4[CO.NH.C(OCH_3)NH]_2$.

Five grams of methyl iso-urea hydrochloride was suspended in moist ether and treated with half its molecular equivalent of phthalyl chloride in the presence of three times its molecular equivalent of potash. The product separated out in a white mass on the sticky residue of potassium hydroxide. It was carefully separated from this residue to avoid contamination with any potassium chloride or free potash and thoroughly washed with ether. It was oily, slightly soluble in ligroin, benzene, and ether, and decomposed in hot alcohol yielding a light yellow oil. Because of its instability in hot solvents, it was not possible to purify it further. Melting point, 112° C. (evolution of gas). Yield, 70% of the theoretical.

Analysis:	N calculated for $C_{12}H_{14}N_4O_4$	20.14%
	Found	20.78, 20.81%

3. *α-Naphthalene-sulphonyl-methyl-iso-urea*, $C_{10}H_7SO_2.NH.C(OCH_3):NH$.

Many acyl derivatives of iso-ureas are oils which are difficult to purify. Various acid chlorides were used as reagents in order to obtain, if possible, some solid, easily purified acyl compounds. α-Naphthalene-sulpho-chloride was used in one or two experiments.

Five grams of methyl iso-urea hydrochloride was suspended in moist ether and treated with its molecular equivalent of α-naphthalene-sulpho-chloride in the presence of twice its molecular equivalent of potash. Part of the product separated out as it had only a moderate solubility in ether, and it was thus necessary to extract the semi-solid residue repeatedly with warm ether. When the ether solution was evaporated, most of the product separated, and the balance was obtained on complete evaporation of the mother liquor. It was soluble in hot alcohol from which it separated, on cooling, in fine white crystals. Melting point, 152° C. Yield, 90% of the theoretical.

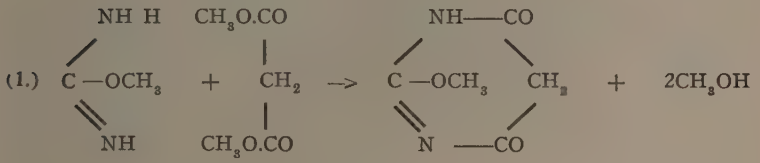
Analysis:	N calculated for $C_{12}H_{12}N_2O_3S$	10.60%
	Found	10.39, 10.27%

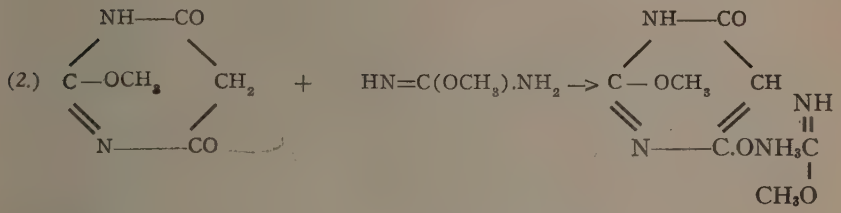
4. *Salt of 2-methoxy-barbituric acid and methyl iso-urea,*

$$N{:}C(OCH_3).NH.CO.CH{:}C.ONH_3C(OCH_3){:}NH.$$

Five grams of methyl iso-urea was mixed with rather more than the equivalent of methyl malonate. The mixture formed an oil which was warmed on the water bath for a few minutes to 60°–70°C. After standing several hours in a vacuum, the oil turned to a white mush of crystals which became semi-solid several days later. Ether was then added and the crystalline masses were broken up with a glass rod. The crystals were separated by filtration and washed with ether. The substance was readily soluble in hot alcohol, from which it separated in fine white crystals. Melting point, 133°C. (with evolution of gas). Yield, 75% of the theoretical.

Analysis: N calculated for $C_7H_{12}N_4O_4$ 25.92%
Found 25.74, 25.55%





5. *2-Methoxy-barbituric acid,* $N{:}C(OCH_3).NH.CO.CH_2.CO.$

Dilute hydrochloric acid (1:10) was added to two grams of the salt described above. The salt was partially dissolved, but a white powder separated immediately. After being allowed to stand for a few minutes, the solid was separated by filtering the mixture, and the substance was washed with cold water to remove the hydrochloric acid. The yield was practically quantitative. The compound was readily soluble in hot alcohol, from which it separated, on cooling, in small white crystals. On being heated, the substance did not melt. It appeared to soften at about 190°C., when it diminished in volume and turned yellow, the color deepening to a reddish tint as the temperature rose to 270°C. This behavior was characteristic of all the barbituric acid ethers described below.

Analysis: N calculated for $C_5H_6N_2O_3$ 19.71%
Found 19.52, 19.60%

That the substance obtained was 2-methoxy-barbituric acid was confirmed by its ready conversion into barbituric acid when boiled with dilute hydrochloric acid. The product obtained decomposed at 250°C. and was identical in every respect with barbituric acid.

Analysis:	N calculated for $C_4H_4N_2O_3$	21.87%
	Found	21.54%

All the barbituric acid ethers were converted by this treatment into barbituric acid.

The acid filtrate from the preparation of 2-methoxy-barbituric acid was evaporated on a water bath. A residue of light yellow oil was left, which slowly crystallized on standing in a vacuum over sulphuric acid and solid potash. It showed on purification a melting point of 132°C., which is that of methyl iso-urea hydrochloride.

SALICYLATE OF ETHYL ISO-UREA, $NH{:}C(OC_2H_5)NH_2.HOOC.C_6H_4.OH$.

This was prepared and purified in the same way as the corresponding methyl compound. Melting point, 153°C. Yield, quantitative.

Analysis:	N calculated for $C_{10}H_{14}N_2O_4$	12.38%
	Found	12.21, 12.32%

n-PROPYL ISO-UREA; SALTS AND ACYL DERIVATIVES

1. *n-Propyl iso-urea hydrochloride*, $NH{:}C(OC_3H_7).NH_2.HCl$.

The method used to prepare this compound was similar to that used in the preparation of methyl and ethyl iso-urea hydrochlorides (4). (See Table I for times of reaction.) The hydrochloride was obtained as a colorless oil which formed long, white, needle-like crystals in ether at −10°C. The crystals on exposure to atmospheric conditions rapidly became moist, and changed to an oil.

2. *Salicylate of n-propyl iso-urea*, $NH{:}C(OC_3H_7).NH_2.HOOC.C_6H_4OH$.

Melting point, 146°C. Yield, quantitative.

Analysis:	N calculated for $C_{11}H_{16}N_2O_4$	11.66%
	Found	11.51, 11.61%

3. *Benzene-sulphonyl-n-propyl iso-urea*, $C_6H_5SO_2.NH.C.(:NH).OC_3H_7$.

Three grams of n-propyl iso-urea was dissolved in moist ether and treated with its molecular equivalent of benzene-sulphonyl chloride in the presence of its molecular equivalent of potash. The product was a light yellow oil, which, on standing in a vacuum, deposited large rhombic crystals. It was readily soluble in hot alcohol, from which it separated, on cooling, in white flaky crystals. Melting point, 74° C. Yield, 90% of the theoretical.

Analysis:	N calculated for $C_{10}H_{14}N_2O_3S$	11.56%
	Found	11.26, 11.25%

4. *Salt of 2-n-propoxy-barbituric acid and n-propyl iso-urea,*

$$N{:}C(OC_3H_7).NH.CO.CH{:}C.ONH_3.C(OC_3H_7){:}NH.$$

The procedure followed in preparing this compound was similar to that used in the preparation of the methyl iso-urea salt of 2-methoxy-barbituric acid. The salt was readily soluble in hot alcohol, from which it separated, on cooling, in fine white crystals. Melting point, 172.5° C. (with evolution of gas). Yield, 60% of the theoretical.

Analysis:	N calculated for $C_{11}H_{20}N_4O_4$	20.58%
	Found	20.40, 20.34%

5. *2-n-Propoxy-barbituric acid,* $N{:}C(OC_3H_7).NH.CO.CH_2CO.$

The procedure followed in preparing this compound was similar to that used in the preparation of 2-methoxy-barbituric acid. The substance was readily soluble in hot alcohol from which it separated, on cooling, in small glistening plates. The yield was practically quantitative.

Analysis:	N calculated for $C_7H_{10}N_2O_3$	16.47%
	Found	16.50, 16.60%

Iso-propyl Iso-urea; Salts and Acyl Derivatives

1. *Iso-propyl iso-urea-hydrochloride,* $NH{:}C[OCH(CH_3)_2].NH_2.HCl.$

The procedure followed in preparing this compound was similar to that used in the preparation of n-propyl iso-urea hydrochloride. (See Table I.) The hydrochloride was obtained as a yellow viscous oil. The latter would not crystallize in ether at −10°C., and remained unchanged after standing in a vacuum for weeks.

2. *Salicylate of iso-propyl iso-urea,* $NH{:}C[OCH(CH_3)_2].NH_2.HOOC.C_6H_4.OH.$

Iso-propyl iso-urea was obtained as an oil on treating the iso-propyl iso-urea hydrochloride in moist ether with four or five times its molecular equivalent of finely ground caustic potash. Three grams of iso-propyl iso-urea in an ethereal solution was treated with its molecular equivalent of salicylic acid dissolved in ether. A few minutes with vigorous stirring were required before the salicylic acid neutralized the iso-propyl iso-urea. The white amorphous substance was very soluble in alcohol, but insoluble in benzene, chloroform and ether. Melting point, 114° C. Yield, quantitative.

Analysis:	N calculated for $C_{11}H_{16}N_2O_4$	11.66%
	Found	11.58, 11.43%

3. *Salt of 2-Iso-propoxy-barbituric acid and iso-propyl iso-urea*, $N{:}C[OCH(CH_3)_2].NH.CO.CH{:}C.ONH_3C[OCH(CH_3)_2]{:}NH.$

The procedure followed in preparing this compound was similar to that used in the preparation of the salt of 2-methoxy-barbituric acid and methyl iso-urea. The salt was slightly soluble in alcohol from which it was recrystallized, but was insoluble in benzene, ether and chloroform. Melting point, 188°C. Yield, 70% of the theoretical.

Analysis:	N calculated for $C_{11}H_{20}N_4O_4$	20.58%
	Found	20.21, 20.25%

4. *2-Iso-propoxy-barbituric acid*, $N{:}C[OCH(CH_3)_2].NH.CO.CH_2.CO.$

The procedure followed in preparing this compound was similar to that used in the preparation of 2-methoxy-barbituric acid. The properties were similar to those of the latter compound. The yield was practically quantitative.

Analysis:	N calculated for $C_7H_{10}N_2O_3$	16.47%
	Found	15.98, 16.06%

n-Butyl Iso-urea; Salts and Acyl Derivatives

1. *n-Butyl iso-urea hydrochloride*, $NH{:}C(OC_4H_9).NH_2.HCl.$

The procedure followed in preparing this compound was similar to that used in the preparation of n-propyl-iso-urea hydrochloride. (See Table I.) The hydrochloride obtained as an oil, crystallized out in ether at −10°C., but immediately turned to a mush of oil and crystals on exposure to the atmosphere.

2. *Salicylate of n-butyl iso-urea*, $NH{:}C(OC_4H_9).NH_2.HOOC.C_6H_4.OH.$

n-Butyl iso-urea was obtained as an oil by treating the n-butyl iso-urea hydrochloride in moist ether with four or five times its molecular equivalent of finely ground caustic potash. Three grams of n-butyl iso-urea in an ethereal solution was neutralized immediately by treating with its molecular equivalent of salicylic acid dissolved in ether. The white amorphous compound crystallized from hot absolute alcohol in fine, white crystals. Melting point, 159° C. Yield, quantitative.

Analysis:	N calculated for $C_{12}H_{18}N_2O_4$	11.01%
	Found	10.84, 10.71%

3. *Salt of 2-n-butoxy-barbituric acid and n-butyl-iso-urea*, $N{:}C(OC_4H_9).NH.CO.CH{:}C.ONH_3C(OC_4H_9){:}NH.$

The procedure followed in preparing this compound was similar to that used in the preparation of the salt of 2-methoxy-barbituric acid and methyl iso-urea. The salt was readily soluble in hot alcohol, from which it separated, on cooling, in fine, white crystals. Melting point, 170° C. Yield, 55% of the theoretical.

Analysis:	N calculated for $C_{13}H_{24}N_4O_4$	18.66%
	Found	18.58, 18.59%

4. *2-n-Butoxy-barbituric acid*, $N{:}C(OC_4H_9).NH.CO.CH_2.CO$.

The procedure followed in preparing this compound was similar to that used in the preparation of 2-methoxy-barbituric acid. The yield was practically quantitative.

Analysis:	N calculated for $C_8H_{12}N_2O_3$	15.21%
	Found	15.03, 15.07%

Iso-butyl Iso-urea; Salts and Acyl Derivatives

1. *Iso-butyl iso-urea hydrochloride*, $NH{:}C[OCH_2.CH.(CH_3)_2].NH_2.HCl$.

The procedure followed in preparing this compound was similar to that used in the preparation of n-propyl iso-urea hydrochloride. (See Table I.) The hydrochloride was obtained as a light brown viscous oil. The latter would not crystallize out in ether at $-10°$C. and remained unchanged when allowed to stand in a vacuum for several weeks.

2. *Salicylate of iso-butyl iso-urea*,

$NH{:}C[OCH_2CH.(CH_3)_2].NH_2.HOOC.C_6H_4.OH$.

Iso-butyl iso-urea was obtained as an oil on treating the iso-butyl iso-urea hydrochloride in moist ether with four or five times its molecular equivalent of finely ground caustic potash. Three grams of iso-butyl iso-urea in an ethereal solution was neutralized immediately by treating with its molecular equivalent of salicylic acid dissolved in ether. The white amorphous compound was readily soluble in hot absolute alcohol, from which it separated, on cooling, in fine white crystals. Melting point, 163° C. Yield, quantitative.

Analysis:	N calculated for $C_{12}H_{18}N_2O_4$	11.01%
	Found	10.84, 10.95%

3. *Salt of 2-iso-butoxy-barbituric acid and iso-butyl iso-urea*,

$N{:}C[OCH_2.CH(CH_3)_2].\ NH.CO.CH{:}C.ONH_3.C[OCH_2.CH.(CH_3)_2]{:}NH$.

The procedure followed in preparing this compound was similar to that used in the preparation of the salt of 2-methoxy-barbituric acid and methyl iso-urea. The salt was readily soluble in hot alcohol, from which it separated, on cooling, in fine white crystals. Melting point, 181°C. The yield was good, but the exact yield was not recorded.

Analysis:	N calculated for $C_{13}H_{24}N_4O_4$	18.66%
	Found	18.40, 18.56%

4. *2-Iso-butoxy-barbituric acid*, $N{:}C[OCH_2.CH(CH_3)_2].NH.CO.CH_2.CO$.

This compound was obtained by the same method as was used for the preparation of 2-methoxy-barbituric acid. The yield was quantitative.

Analysis:	N calculated for $C_8H_{12}N_2O_3$	15.21%
	Found	14.98, 15.01%

Iso-amyl Iso-urea; Salts and Acyl Derivatives

1. *Iso-amyl iso-urea hydrochloride*, $NH:C[OCH_2.CH_2.CH.(CH_3)_2].NH_2.HCl$.

The procedure followed in preparing this compound was similar to that used in the preparation of N-propyl iso-urea hydrochloride. (See Table I.) The hydrochloride was obtained as a light yellow, viscous oil. The latter would not crystallize out in ether at $-10°C$. and remained unchanged when allowed to stand in a vacuum for several weeks.

2. *Salicylate of iso-amyl iso-urea*,
$NH:C[OCH_2.CH_2.CH(CH_3)_2].NH_2.HOOC.C_6H_4.OH$.

Iso-amyl iso-urea was obtained as an oil on treating the iso-amyl iso-urea hydrochloride in moist ether with four or five times its molecular equivalent of finely ground caustic potash. Three grams of iso-amyl iso-urea in an ethereal solution was neutralized almost immediately by treating with its molecular equivalent of salicylic acid dissolved in ether. The white amorphous compound was readily soluble in hot absolute alcohol from which it separated, on cooling, in small white crystals. Melting point, 155°C. Yield, quantitative.

Analysis:	N calculated for $C_{13}H_{20}N_2O_4$	10.43%
	Found	10.24, 10.31%

3. *Salt of 2-iso-amoxy-barbituric acid and iso-amyl iso-urea*,
$N:C[OCH_2.CH_2.CH.(CH_3)_2].NH.CO.CH:C.ONH_3.C[OCH_2.CH_2.CH.(CH_3)_2]:NH$.

The procedure followed in preparing this compound was similar to that used in the preparation of the salt of 2-methoxy-barbituric acid and methyl iso-urea. The salt was readily soluble in hot alcohol, from which it separated, on cooling, in small white crystals. Melting point, 165° C. Yield, 80% of the theoretical.

Analysis:	N calculated for $C_{15}H_{28}N_4O_4$	17.06%
	Found	17.03, 17.01%

4. *2-Iso-amoxy-barbituric acid*, $N:C[OCH_2.CH_2.CH(CH_3)_2].NH.CO.CH_2.CO$.

The procedure followed in preparing this compound was similar to that used in the preparation of 2-methoxy-barbituric acid.

Analysis:	N calculated for $C_9H_{14}N_2O_3$	14.13%
	Found	13.83, 13.93%

Benzyl Iso-urea

1. *Benzyl iso-urea hydrochloride*, $NH:C(OCH_2.C_6H_5).NH_2.HCl$.

The preparation of this compound was carried out as follows: 3.5 gm. of dry hydrogen chloride was passed into a solution of 4 gm. of dry cyanamide and 10.3 gm. of benzyl alcohol in 96 gm. of ethylene chlorhydrin. The

latter was used as a solvent because it does not react with cyanamide and can be distilled off in a vacuum without the decomposition of the benzyl-iso-urea hydrochloride. No di-hydrochloride of cyanamide separated out and the mixture showed no reaction for cyanamide after being allowed to stand for a period of eight days. The ethylene chlorhydrin was distilled off under reduced pressure. This left the hydrochloride as an oil which would not crystallize in ether at −10°C.

2. *Salicylate of benzyl iso-urea*, $NH:C(OCH_2C_6H_5).NH_2.HOOC.C_6H_4.OH$.

Benzyl iso-urea was obtained as an oil on treating the iso-urea hydrochloride in moist ether with four times its molecular equivalent of finely ground caustic potash. An ethereal solution of benzyl iso-urea was treated with its molecular equivalent of salicylic acid dissolved in ether. The salicylate was deposited as a white amorphous substance almost immediately. It was soluble in hot alcohol from which it separated in fine white crystals. Melting point, 140°C. Yield, almost quantitative.

Analysis:	N calculated for $C_{15}H_{16}N_2O_4$	9.72%
	Found	10.19, 10.19%

Phenyl-ethyl Iso-urea

1. *Phenyl-ethyl iso-urea hydrochloride*, $NH:C(OCH_2.CH_2C_6H_5).NH_2.HCl$.

The procedure followed in preparing this compound was that used in the preparation of benzyl iso-urea hydrochloride. No di-hydrochloride of cyanamide separated and the reaction for cyanamide disappeared in seven days. The ethylene chlorhydrin was distilled off under reduced pressure and the hydrochloride was left as a mush of oil and crystals which would not solidify in ether at −10°C.

2. *Salicylate of phenyl-ethyl iso-urea*,

$NH:C(OCH_2.CH_2.C_6H_5).NH_2.HOOC.C_6H_4.OH$.

An ethereal solution of phenyl-ethyl iso-urea was treated with its molecular equivalent of salicylic acid dissolved in ether. The salicylate was deposited as a white amorphous substance almost immediately. After being thoroughly washed with ether, it was found to be very soluble in alcohol, but only slightly soluble in benzene, ether, chloroform and ligroin (b.p. 60° to 90° C.). Melting point, 131° C.

Analysis:	N calculated for $C_{16}H_{18}N_2O_4$	9.27%
	Found	8.94, 8.94%

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STUDIES ON THE ACTION OF SULPHATES ON PORTLAND CEMENT

I. THE USE OF THE EXPANSION METHOD IN THE STUDY OF THE ACTION OF SULPHATES ON PORTLAND CEMENT MORTAR AND CONCRETE[1]

By T. Thorvaldson[2], D. Wolochow[3] and V. A. Vigfusson[3]

Abstract

This paper describes the methods employed in the use of expansion measurements as a means of studying the action of sulphates on Portland cement, and on Portland cement mortars. Experimental data are given dealing with the reproducibility of the expansion measurements and the relation between expansion and loss of tensile strength of mortars. Results obtained with standard sand mortars and graded sand mortars of varying richness of mix prepared from cements which differ in their resistance to sulphate action are presented.

Introduction

The method in common use for testing the quality of Portland cements, or for following any changes which they or materials made from them undergo, involves the determination of the tensile or compressive strength of suitable mortar or concrete specimens. Experience has shown that, even with test pieces made and cured under ideal conditions, considerable variations are found in the results. This makes it necessary to use several specimens for each experimental value required. When this method is applied to the study of the effect of conditions which have a weakening or disintegrating action on the mortar or concrete, it is found that the variations in strength for exposure of similar test pieces under apparently identical conditions are materially increased. This necessitates the use of still greater numbers of test pieces in order to obtain reliable average results. Since a specimen is destroyed in making the test, the number required in the study of a problem involving several factors soon becomes so excessively large as to introduce other difficulties, for instance, the maintenance of uniformity of materials and conditions over the whole investigation. This applies especially to a study of the influence of various conditions on the resistance of a mortar or concrete to the action of alkali waters, where the effect of factors such as the cement used, the richness of mix, the kind of sulphate (and other salts) present in the alkali water, and the concentration of each must be considered for each condition. For these reasons the development of new, reliable quantitative methods for the study of the action of alkali waters on mortars or concrete would be of great importance.

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Contribution from the laboratories of the University of Saskatchewan, Saskatoon, Canada, with financial assistance from the National Research Council of Canada. This paper is the first of a series.
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A physical method, based on the expansion of mortars and concrete during the action of sulphate waters upon them, has been developed independently in two different laboratories. In the spring of 1922 an attempt was made in the chemical laboratory of the University of Saskatchewan to obtain a measure of the action of sulphate solutions on the colloid gel in Portland cement mortars by determining how the swelling of the mortar in water is affected by exposure to such solutions. The predetermined plan was to measure the linear expansion on wetting in water, and the contraction on drying, of mortar bars which had been exposed for different lengths of time to the action of the sulphate solution. At the same time the approximate weight of water absorbed and given up was to be determined. It was found that when mortar bars of lean mixes were exposed to sulphate solutions they expanded in a very regular manner until the action of the sulphate caused bending or cracking of the specimens. The method of determining the rate of linear expansion was at once applied to a study of the effect of various factors on the progress of the action of sulphates on mortars (5, 7, 8, 9). A similar method, using test pieces of concrete and measuring their rate of linear expansion in alkali water, was developed by Dalton G. Miller at the Drain Tile Laboratory of the University of Minnesota. The results of a number of these studies have been published by this author (1, 2, 3, 4).

It is evident that the expansion method, if applicable to the exact study of the action of sulphate waters on Portland cement, avoids one of the chief disadvantages of the tensile or compressive strength method, namely, the necessity for using a very large number of specimens. A test piece is not destroyed in determining the increase in length, but can be used as long as the action of the sulphate leaves it intact. Thus the whole expansion curve can be obtained with the same number of test pieces as are required for the determination of a single point on the strength curve. This economy is of very great importance when working with small quantities of materials made in the laboratory.

The examination of concrete structures which have failed on account of the action of alkali water clearly indicates, in most cases, that uneven expansion has played an important part in causing the failure. There is scarcely ever a condition of uniform action of the sulphate over the whole structure. As the action of the sulphate on the concrete progresses, a disruptive stress is developed by the tendency of a portion of the mass to expand. This stress increases as the action goes on. At the same time, the strength of the concrete decreases through the action of the sulphate. Ultimately the stress exceeds the strength, cracks are formed, and it is considered that failure has occurred. Thus one comes to the conclusion that in the failure of a structure, expansion is usually just as important a factor as loss of strength. A complete record of the progress of sulphate action can hardly be obtained unless both the decrease in strength and the stress tending to cause deformation can be measured. The expansion of an unconfined test piece which is immersed in a sulphate solution depends both on the expansive force caused by

the action of the sulphate and on the cohesive strength of the specimen. It might therefore be expected that the determination of the expansion of the mortar would be a good measure of the progress of sulphate action. To a large extent this has been found to be true of normal Portland cement mortars and concrete.

The same assumption must not be made when experimenting with mortars or concrete made of Portland cement which has been modified either by changing the composition of the cement or by treatment of the material. It should be remembered that Portland cement is a complex product containing several chemical components and that the three sulphates generally present in alkali water show a certain individuality when acting on cement mortars. It is therefore possible that the two factors, the tendency to expand and the loss in strength in sulphate solutions, may be affected independently. Thus a given treatment might prevent expansion and yet might not prevent some chemical change which would weaken the specimen; on the other hand, expansion might take place with an increase or very small decrease in the strength of the specimen. In either case, failure might result. Such cases have been observed in this laboratory.

Experimental Procedure

That it is very difficult to prepare ordinary test pieces of mortar or concrete which will give uniform results for compressive and tensile strength tests, is well known. The specimens used in this laboratory for determining the expansion curves are very small and hence present even greater difficulties during both preparation and curing. If the experiments are to be comparable, all the work of preparing, curing, and exposing the test pieces to the sulphate solutions must be done at constant temperature. The control of atmospheric humidity during preparation of the specimens is also very important. As the amount of surface of experimental mortar bars is large in proportion to their weight, it is of the greatest importance that the air in the damp chamber in which the bars are stored during the period of curing should be continually saturated with water vapor; the drying out of the specimens, after the hydration of the cement is reasonably advanced, has a very large effect on their resistance to the action of sulphates. It may therefore be easier to secure comparative results by curing the bars in distilled water in sealed jars, when this is feasible.

The bars used for the expansion measurements are rectangular prisms 0.625 in. thick, and of two different lengths, 7.5 in. and 10 cm.(3.937 in.). In order to avoid, as far as possible, slight variations in sand and water, standard Ottawa sand and distilled water are used. The quantities of all components are determined by weight. In the early work in this laboratory, attempts were made to determine by means of the flow table the amount of mixing water required in each case. This introduced difficulties with the leaner mixes, hence the method was changed so as to use, for 1:3 or leaner mortars, the amount of water called for by the standard specifications for a

1:3 standard sand mix. After the cement and sand have been thoroughly mixed, the water is added and the whole mass well worked to ensure uniformity. The specimens are then moulded in collapsible steel frames. When the moulds are being filled, the tamping and troweling are done as mechanically as possible in order to obtain uniform specimens. The moulds are then stored in a damp closet until the bars are strong enough to be removed, the time required varying with the richness of the mortar. The bars are then cured in a damp closet or in distilled water in sealed jars until exposed to the sulphate solutions or to other treatment.

The mortar briquets used for the corresponding tensile strength determinations are made up according to the standard specifications for Portland cement, the mix and the storage treatment being the same as for the corresponding mortar bars. The determinations of tensile strength are made in accordance with the standard specifications.

Considerable difficulty was at first experienced in obtaining bars with ends suitable for accurate measurement of length. The ends must be smooth, free from easily detachable material, and more resistant to the action of sulphates than the bar itself. After considerable experimentation, the method finally adopted was to make the bars with a thin layer of neat cement on each end. This gave a suitable surface which enabled reproducible measurements of length to be made.

The mortar bars have been found to be well suited to the study of expansion during the action of sulphate solutions. They possess sufficient strength for handling except where the mortar is too lean, and are long enough to permit the measurement of even a slight expansion. The lean mixes allow very rapid penetration of solutions throughout the whole mass.

Measurements of the length of the bars are made by means of a micrometer head set in a steel frame. The micrometers used are graduated to read to 0.0001 in. and to 0.01 mm., respectively.

After the initial measurements at the end of the curing period have been made, the bars are placed in the sulphate solution in glass sealers of suitable size, and are measured as often as necessary for the determination of the expansion curve. All the work is carried out in a constant-temperature room, the temperature of which rarely varies more than 0.2° from 21°C.

Reproducibility of the Expansion Curve

The cause of the expansion of Portland cement mortars in sulphate solutions is not known with certainty. The expansion of mortars in pure water is, without doubt, caused by swelling of the colloid gel which is formed mainly from the calcium silicates of the cement through the process of hydrolysis or hydration. It has been shown (6) that the hydrolysis of the silicates in cement proceeds further in solutions of sulphates than in pure water, and it might therefore be expected that this fact was partly responsible for the increased expansion. It is, however, generally considered that the expansion is due to the formation, with increase in volume, of crystals of calcium sulphoaluminate

and calcium sulphate in the mortar through the action of the sulphate on the aluminate and the free lime in the hydrated cement. But whether expansion is caused by pressure due to the swelling of a colloid gel or is due to the pressure exerted by growing crystals, considerable variability would be expected in a material such as cement mortar. For this reason strict reproducibility of the time-expansion curve is not to be expected.

It was found that variations in the treatment of the mortar bars during the curing period affected their rate of expansion in solutions of sodium sulphate or calcium sulphate much more than in solutions of magnesium sulphate (7). In investigating the reproducibility of the expansion curve a 0.15 M. solution of sodium sulphate was therefore selected. Six batches of bars made of one part of Portland cement to 7.5 parts of standard Ottawa sand were prepared on different days. They were cured for seven days in the moulds. One-half of each batch were then cured in distilled water, the other half in a damp closet, until immersed in the sulphate solution. After curing periods of 14 days, one month, and three months, three bars from each of the six batches were placed in a sealer containing 1,700 cc. of the sulphate solution. Each value given in Table I for time of expansion is therefore the average for three bars.

TABLE I

COMPARISON OF THE RATE OF EXPANSION OF MORTAR BARS MADE ON DIFFERENT DAYS*

Linear expansion per cent	Time in days to produce given expansion						
	Batch No. 162	Batch No. 163	Batch No. 164	Batch No. 165	Batch No. 166	Batch No. 167	Average
A. Bars cured 14 days in damp closet							
0.01	4.3	5.0	5.6	5.3	5.7	4.8	5.1
0.02	6.5	7.1	7.1	6.9	7.2	6.6	6.9
0.05	8.7	8.5	8.4	8.8	9.1	8.8	8.7
0.10	10.0	9.6	9.3	10.1	10.4	10.0	9.9
0.20	11.2	10.8	10.4	11.3	11.6	11.3	11.1
0.50	12.8	12.4	12.2	13.1	13.2	13.0	12.8
1.00	14.3	13.7	13.7	14.5	14.8	14.5	14.3
2.00	15.9	15.3	15.0	16.5	16.6	16.2	15.9
B. Bars cured seven days in damp closet and seven days in water							
0.01	5.9	5.6	6.8	5.4	5.5	5.7	5.8
0.02	7.2	7.2	8.0	7.0	7.2	7.4	7.3
0.05	9.0	9.2	9.3	8.6	9.2	8.4	9.0
0.10	10.1	10.5	10.7	9.8	10.5	9.7	10.2
0.20	11.3	11.8	11.8	11.0	11.8	11.0	11.5
0.50	13.0	13.6	13.8	12.7	13.5	12.7	13.2
1.00	14.7	15.3	15.5	14.1	15.4	14.3	14.9
2.00	16.5	17.1	17.6	15.7	17.5	16.5	16.9

**Mix: One part of cement to 7.5 parts of standard sand.*

The average difference between the low and the high value for each of the eight expansions is slightly more than one day, for both methods of curing. The largest fluctuations occur in the time for the lowest (0.01%) and the highest (2%) expansion.

An exactly corresponding series of experiments was made with bars cured one month and three months. The average difference between the highest and the lowest number of days obtained for the eight expansions was one day for the bars one month old and 1.5 days for the bars three months old. As in the case of the 14-day bars, the largest fluctuations were found at very low and very high expansions.

The results shown in Table I are typical of those generally obtained with the method. Occasionally, however, it is found that, without any known cause, more widely varying results are obtained. With solutions of magnesium sulphate the variations are smaller, and large differences are less frequent than with solutions of sodium sulphate.

These variations between repeated determinations are small when compared with the corresponding variations obtained with Portland cement from different sources. Table II gives the results obtained with three Portland cements, each from a different mill. All the cements conformed to the requirements of the standard specifications.

TABLE II

RELATIVE EXPANSION OF PORTLAND CEMENT MORTARS IN SULPHATE SOLUTIONS*

Linear expansion per cent	Time in days to produce given expansion		
	Cement No. 127	Cement No. 326	Cement No. 826
1. In 0.15 M. Na_2SO_4			
0.01	1	4	3
0.02	3	6	6
0.05	5	7	10
0.10	6.5	8	14
0.20	7.5	9	20
0.50	9	11	30
1.00	10.7	13	42
2. In 0.15 M. $MgSO_4$			
0.01	1	2	1
0.02	1	3.5	3
0.05	3	5.5	7
0.10	4	7	9
0.20	5	9	12
0.50	6.5	11	18
1.00	9.5	14	33

**Mix: One part of cement to 10 parts of standard sand.*

NOTE: *Bars were cured 15 days before exposure to sulphate solutions.*

Relation between Expansion and Tensile Strength

Curves showing the changes in tensile strength of 1:5 standard-sand briquets and the corresponding changes in the length of standard bars of the same composition when exposed to a 0.15 M. solution of sodium sulphate have been published elsewhere (7). Some uncertainty was introduced into the comparison on account of the difference in cross section of the bars and the briquets. A special clamp was therefore devised which made it possible to determine the expansion and the tensile strength on the same mortar specimen. The clamp, with the bar to be tested held in it, is of the exact shape and size of a standard briquet. For the determination of tensile strength, it can thus be inserted into the standard clip of a cement tester.

The results given in Fig. 1 were obtained with 1:5 standard-sand mortar bars made from a normal Portland cement. The bars were cured in the moulds in a damp closet for seven days and in water for an additional seven days before being exposed to the sulphate solutions. The solutions of 0.50 M. Na_2SO_4 and 0.50 M. $MgSO_4$ were used.

The tensile strength of the bars increases very rapidly during the first two days of immersion in the sulphate solutions and then remains practically constant for several days. During the latter period there is very little expansion, and bars immersed in distilled water gradually overtake in strength those exposed to the sulphate solutions. With an increase in the concentration of the solutions, the length of this period of high strength and very slight expansion decreases. A critical point seems then to be reached, and a very rapid decrease in tensile strength takes place; at the same time, the rate of expansion begins to increase. The expansion-strength curve is not linear, the tensile strength diminishing at a decreasing rate with respect to expansion.

Relation between the Expansion of Lean and Rich Mortars Made from the Same Cement

Very lean mortars are generally used in this laboratory for the study of the action of sulphate solutions on cements by the expansion method since, due to their porosity, they allow free access of the solution to the cement particles throughout the whole mass. The effect of the sulphate solutions upon the cement constituent can thus be rapidly determined, and variations, due to decreased permeability in the richer mixes, can be avoided.

The use of chemical methods for determining the stability of cements in sulphate solutions presents serious difficulties, especially in the interpretation of results. Normal hydration of cement in water causes hydrolysis of the silicates, while exposure to sulphate waters speeds up this process and carries it further. The point where beneficial hydrolysis ceases and harmful hydrolysis begins is not definitely known. On the other hand, while the microscope is of great assistance in the study of the action of sulphates on cement, its use can hardly be made quantitative except in determining certain limiting conditions.

The expansion method, using lean mortars, has the merit of being rapid and of giving quantitative results in terms of a factor related to the stability of the material in which Portland cement is used, namely the expansion—and indirectly the strength—of the mortar or concrete. When dealing with very lean standard-sand mortars, which are freely permeable to the solutions, it might be expected that the relative expansion of mortars from different cements would be a measure of the relative stability of the cements in the sulphate solution used. If this be granted, there is still the question whether the expansion of mortar made from rich mixes, such as good concretes of low permeability, is a measure of their stability. The time required for disintegration of a bar of 1:10 sand mortar in 0.15 M. Na_2SO_4 is measured in days, and it might be suggested that the difference between the 10 and 40 days required for attaining a linear expansion of 1% (Table II) may have very little significance when applied to good concretes made from these cements. On the other hand, it takes four times as long for 1:10 mortar bars made of cement No. 826 as for similar bars of cement No. 127 to expand 1%, and this ratio may be expected to remain constant or to increase in magnitude for less permeable mixes of the two cements.

A series of experiments was therefore made on the relation of expansion to the richness of the mortar. Using both standard sand and a well graded sand as aggregate, test pieces of two cements were made, the mixes being 1:10, 1:5, 1:3 and 1:2. Both were normal Portland cements conforming to the standard specifications. In each case, the normal consistency was 23.5%. The amount of mixing water used was calculated according to the formula:

$$\text{Per cent water} = \frac{2}{3} \cdot \frac{P}{n+1} + K$$

where P = per cent water for neat normal consistency,
K = 6.5,
n = parts of normal sand to one part of cement.

The composition of the sand (Warman No. 430) used in the preparation of the graded-sand mortars is given in Table III. The test pieces were exposed to 0.15 M. and 0.50 M. solutions of both sodium sulphate and magnesium sulphate, and measurements of length were made until the expansion in each case had reached 0.5% of the length of the specimen.

The results for the standard sand mortar in 0.15 M. Na_2SO_4 are given in Table IV.

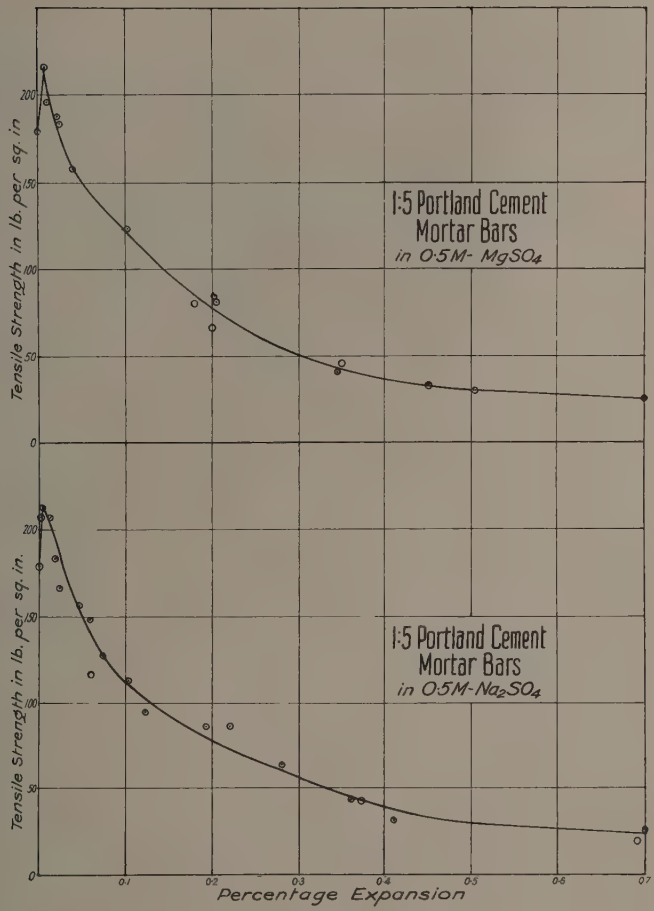

FIG. 1. *Relation between Expansion and Tensile Strength*

TABLE III

SCREEN ANALYSIS OF GRADED SAND (WARMAN NO. 430)*

Mesh per inch	Percentage weight	Cumulative percentage weight
On 8	24.9	24.9
" 14	27.1	52.0
" 28	28.5	80.5
" 48	12.0	92.5
" 100	3.7	96.2
Through 100	3.8	100.0

NOTE: *The density of the sand = 102 lb. per cu. ft. and the surface area = 470 sq. in. per 100 grams.*

**This sample of sand and the report on granular analysis were kindly supplied by the Civil Engineering Laboratories of the University of Saskatchewan.*

TABLE IV

EXPANSION OF MORTAR BARS IN 0.15 M. SODIUM SULPHATE SOLUTIONS

Mix: Ratio of cement to standard sand	Time in days					
	Expansion 0.05	Expansion 0.10	Expansion 0.20%	Expansion 0.50	Expansion, sum	Expansion ratio Mix 1:10 = 1
Cement No. 427						
1:10	4.8	6.1	7.5	9.2	27.6	1.00
1:5	6.5	8.5	10.0	12.3	37.3	1.35
1:3	17.8	23	27.3	31.8	99.9	3.62
1:2	52.5	78	97	125	352.5	12.77
Cement No. 128						
1:10	6.8	7.5	8.5	10.0	32.8	1.00
1:5	12.8	14.8	17.7	19.2	64.5	1.92
1:3	38	55	71	89	253	7.73
1:2	106	252	351	450	1159	35.34

The expansion ratios in the last column of Table IV may be assumed to represent the relative resistance of the various mixes of each cement to the action of 0.15 M. solutions of sodium sulphate. On this assumption, it is evident, first that the resistance for each cement increases rapidly with increasing richness of mix; and, second, that the resistance of cement No. 128, which in a 1:10 mix is slightly more resistant than No. 427, increases more rapidly than does that of No. 427.

The relative resistance of mortars of the same mix for the two cements may be determined by calculating the ratio between corresponding figures in the "sum" column of Table IV, as follows:

Mortar (cement to sand): 1:10; 1:5; 1:3; 1:2.

$$\text{Ratio} : \frac{\text{cement No. 128}}{\text{cement No. 427}} : 1.19; 1.73; 2.53; 3.30.$$

Thus the superiority of cement No. 128, as indicated by the results of the exposure of 1:10 mortar, becomes much greater with increased richness of mix.

Tables V, VI and VII give a summary of the results obtained in this way with cements No. 128 and 427 in mortars made with standard sand and with graded sand (Warman No. 430).

TABLE V

RELATIVE RESISTANCE OF STANDARD SAND MORTARS TO SULPHATE ACTION AS CALCULATED FROM EXPANSION MEASUREMENTS*

Mix: Ratio of cement to standard sand	Cement No. 427				Cement No. 128			
	Na_2SO_4		$MgSO_4$		Na_2SO_4		$MgSO_4$	
	0.15 M	0.50 M	0.15 M	0.50 M	0.15 M	0.50 M	0.15 M	0.50 M
1:10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1:5	1.35	1.21	1.14	2.15	1.92	1.34	1.99	2.74
1:3	3.62	3.28	3.27	7.25	7.73	3.85	6.78	9.36
1:2	12.77	13.13	8.32	24.90	35.34	17.49	13.57	21.86

**Resistance of 1:10 mortar taken as unity.*

TABLE VI

RELATIVE RESISTANCE OF GRADED SAND MORTARS TO SULPHATE ACTION AS CALCULATED FROM EXPANSION MEASUREMENTS*

Mix: Ratio of cement to standard sand	Cement No. 427				Cement No. 128			
	Na_2SO_4		$MgSO_4$		Na_2SO_4		$MgSO_4$	
	0.15 M	0.50 M	0.15 M	0.50 M	0.15 M	0.50 M	0.15 M	0.50 M
1:10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1:5	1.77	1.81	1.98	1.65	2.62	2.06	2.53	2.39
1:3	4.75	4.04	5.95	8.00	10.88	5.20	7.77	9.36
1:2	8.15	7.56	8.84	16.40	22.88	13.11	12.60	13.07

**Resistance of 1:10 mortar taken as unity.*

TABLE VII

RELATIVE RESISTANCE OF CEMENTS No. 128 AND 427 TO SULPHATE ACTION AS CALCULATED FROM EXPANSION MEASUREMENTS ON MORTAR BARS*

Mix: Ratio of cement to standard sand	Standard Sand				Graded Sand			
	Na_2SO_4		$MgSO_4$		Na_2SO_4		$MgSO_4$	
	0.15 M	0.50 M	0.15 M	0.50 M	0.15 M	0.50 M	0.15 M	0.50 M
1:10	1.19	1.88	1.27	1.24	1.92	2.23	1.80	1.40
1:5	1.73	1.91	1.64	1.58	2.84	2.55	2.36	2.03
1:3	2.53	2.21	1.86	1.60	4.41	2.88	2.35	1.64
1:2	3.30	2.72	1.53	1.09	5.40	3.88	2.03	1.12

**Resistance of mortars of cement No. 128 in terms of corresponding mortar of cement No. 427 as unity.*

Tables V and VI show how the resistance to various sulphate solutions increases with the richness of mix in the case of the two cements, in the form of mortars of both standard and graded sand. From inspection of the results, it may be seen that the rise in resistance with increasing content of cement with both sands is much more rapid for mortars from cement No. 128 than from No. 427, the only exception being the 1:2 mortar in 0.5 M. $MgSO_4$. From Tables V and VI it is clear that the resistance increases faster with graded sand up to and including the 1:3 mortar, but that the increase in resistance with a 1:2 mortar is greater for the standard sand.

From an examination of Table VII it may be seen that the superior resistance to sulphate action of cement No. 128 over No. 427, as expressed by the relative time necessary for test pieces to attain a given expansion in solutions of sodium sulphate, is not only maintained but increases with the richness of the mortar. In magnesium sulphate solutions the ratio at first increases with the richness of mortar but diminishes in very rich mixes. This is especially noticeable in the 0.50 M. solution. This does not mean that the resistance of the mortar to concentrated solutions of magnesium sulphate decreases with rich mixes, but that the difference between the resistance of the two cements decreases.

A comparison of the data in Table VII for standard and graded sand shows that the numerical ratios are larger for the graded sand. This indicates that the superiority of cement No. 128 over No. 427 is more marked with graded-sand mortar or concrete than with standard-sand mortar.

Similar work done with other cements, of varying resistance to the action of sulphate solutions, has given results in agreement with the above data. Thus it has always been found that of any two cements, that one showing the greater resistance in a 1:10 mortar also develops resistance more rapidly as the richness of mix is increased. For example, if one cement shows twice the resistance of another in the form of 1:10 mortar, the expansion data indicate that the life of a good concrete made from this cement will be considerably more than twice that of a similar concrete made from the less resistant cement.

Determinations of the corresponding changes in tensile or compressive strength of specimens of the different mixes have not been carried out systematically. The data at hand, however, indicate that, when dealing with cements differing in sulphate resistance, the variations in the relative time required for loss of tensile strength of mortar briquets are similar to those occurring in the expansion experiments. Very extensive experiments would be necessary in order to obtain a detailed comparison. The importance of expansion in an investigation of the stability of Portland cement mortar or concrete in sulphate solutions is, however, so great as to warrant a careful study of this factor, independent of changes in the strength of the material.

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